"FORMULATION AND EVALUATION OF PRONIOSOME BASED NIOSOME OF BOSENTAN MONOHYDRATE FORORAL DELIVERY"

Dissertation submitted to

THE TAMILNADUDr.M.G.R.MEDICALUNIVERSITY,

CHENNAI – 32.

In partial fulfillment of the requirements for the award of the degree of

MASTER OF PHARMACY

IN

PHARMACEUTICS

Submitted by

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This is to certify that the works embodied in this dissertation entitled **"*FORMULATION AND EVALUATION OF PRONIOSOME BASED NIOSOME OF BOSENTAN MONOHYDRATE FOR ORAL DELIVERY*"** submitted in the partial fulfillment for the degree of **MASTER OF PHARMACY** in Pharmaceutics, The Tamil Nadu Dr. M.G.R. Medical University, Chennai, is a bonafide work, which was carried out by**Mr.KHILANAND (Reg.No.261210807)**under my guidance and supervision during the academic year 2013-2014.

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DECLARATION

The work presented in this dissertation entitled ***"FORMULATION AND EVALUATION OF PRONIOSOME BASED NIOSOME OF BOSENTAN MONOHYDRATE FOR ORAL DELIVERY"***was carried out by me under the guidance of**Mrs.S.CHANDRA., M.Pharm., (Ph.D).,** AssistantProfessor in Department of Pharmaceutics, J.K.K.Munirajah Medical Research Foundation College of Pharmacy, Komarapalayam.This work is original and has not been submitted in part or full for the award of any other degree or diploma of any other university.

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ACKNOWLEDGEMENT

No research is ever the outcome of single individual's talent or efforts. I have seen and experienced the countless blessing showered on me by my parents, all family members, teachers, friends and all my well-wishers knowing the God's hand is there, always guiding me and leading me to greater heights. It provides me pleasure to convey my gratitude to all those who have directly or indirectly contributed to make this work a success.

Teacher is a guide, philosopher and friend, which I have experienced in my respected guide ***S. Chandra., M. Pharm, (PhD)., Asst. Professor,*** *Department of Pharmaceutics, with deep sense of gratitude. I heartily thank her for providing unceasing encouragement, precious and erudite suggestions, directions and guidance along with the freedom of work that she gave me. To work under her guidance has been a great experience.*

I take this opportunity to express my sincere gratitude and thanks to Prof. ***K. G. Parthiban.,*** *M. Pharm., (Ph.D)., Head of the Department, Department of pharmaceutics, for his valuable guidance and keen interest shown towards my work.*

It is indeed my proud privilege to express my deep sense of gratitude and indebtedness to our principal, ***Dr. N. Senthil Kumar****., M. Pharm., Ph.D, JKKMMRF'S Annai JKK Sampoorani Ammal College of Pharmacy for providing me the necessary facilities and amenities to carry out my dissertation work effectively.*

I express my deepest sense of gratitude to our beloved chairperson and managing trustee ***Dr. J.K.K. Munirajah, M.Tech., (Bolton),*** *for the facilities provided at the institution enabling me to do work of this magnitude.*

Words are not enough to express my sincere and humble thanks to the faculty members ***Dr. R. Manivannan****.,* ***Mr. Krishnarajan****.,* ***Mr. Vijay Amruthraj****., and* ***Mr. J. Ramesh****., for their support and help throughout the course..*

Words are not enough to express my sincere and humble thanks to ***Dr. M.S. Karchuli., Mr. Saurabh Jain and Mr. Amit Nayak*** *for providing gift sample of drug and helping throughout the completion of this dissertation successfully.*

My heartful thanks to my classmates and friends ***Miss. Rajeswari, Mr. Ravi Kumar, Miss. Nidhi Dixit, Miss. Madhumita Sangar, Mr. Saleem, Miss. Sangeetha Thakur, Miss. Romana Malik, Mr. Gopala Rao, Mr. K. Naveen, Mr. Rajesh. G., Mr. Shyam,*** *for helping and encouraging me throughout the completion of the project.*

I thank to ***Mrs. Kasturi,*** *lab technician,* ***Mrs. Rajamani,*** *Librarian, and* ***Mr. Saravanan,*** *Store Keeper for their constant help in the research work.*

Finally I consider this as an opportunity to express my gratitude to all the dignitaries who have been involved directly or indirectly with the successful completion of this dissertation.

KHILANAND

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INTRODUCTION

In the last few decades, much research has been aimed at prevention and treatment of disease with a significant degree of success. Treatment of an acute disease or a chronic illness has been accomplished by administering various pharmaceutical dosage forms like tablets, capsules, creams ointments, syrups, suppositories and injectables to the patients. Even though these dosage form ensure a prompt release of drug, it is necessary to administer these conventional dosage form several times a day to maintain the drug concentration within the therapeutically effective range for the treatment.

In the past three decades several advancement in drug delivery system have been made. As a result new techniques have been developed as delivery systems. These techniques are capable of controlling the rate of drug, delivery. Sustaining the duration of therapeutic activity and targeting the delivery of a drug to a tissues.

New carrier system has been designed to procure site-specific pharmacological action drug or controlled release of drug or prolonged duration of action of the drug, thus enhancing efficacy while diminishing undesirable side effects.

Novel drug delivery system are being developed rapidly so as to overcome the limitations of conventional dosage form.

Improving the effectiveness of an existing drug by optimizing the delivery and dosage, minimizing the side effect and finding the therapeutic uses may be a better investment and more effective for patients than creating a brand new drug. The objective is to encourage research to develop technologies for targeted controlled and sustained delivery of drug with reduced side effects that efficiently deliver drugs into cells.

NOVEL DRUG DELIVERY SYSTEM

The goal of any drug delivery system is to provide a therapeutic amount of drug to proper site in the body to maintain the desired drug concentration. That is the drug delivery system should deliver the drug at a rate dedicated by the needs of the body over a specified period of treatment.

The development of several novel drug delivery system of medication provides a number of therapeutic benefits. The novel drug delivery system may be broadly grouped into two types namely:

- Spatial drug delivery system and
- Temporal drug delivery system

1. Spatial drug delivery system:

It relates to targeting a drug to a specific organ or tissue.

2. Temporal drug delivery system:

It relates to controlling the rate of drug delivery to the target tissue

Present scenario and future of NDDS:

In India major thrust area of research and development for NDDS are:

- Liposomes
- Niosomes
- Nanoparticles
- Transdermal drug delivery
- Implants

- Oral system
- Microencapsulation or microcapsules
- Polymer in drug delivery

Novel drug delivery system can be broadly divided into two classes. They are as follows

- ❖ Sustained release drug delivery system
- ❖ Controlled release drug delivery system

Sustained release drug delivery system includes any drug delivery system that releases the drug over an extended period of time. If the system is successful in maintaining constant drug level in the drug or target tissue, it is considered as a controlled release system. If it extends only the duration of action over the conventional delivery system, it is considered as prolonged release system

SUSTAINED RELEASE DRUG DELIVERY SYSTEM

Sustained release drug delivery system is described as a pharmacological dosage form, formulated to retard the release of a therapeutic agent such that its appearance in the systemic circulation is delayed and / or prolonged and its plasma profile is sustained in duration. The onset of its pharmacological action is often delayed and its duration of the therapeutic effect is extended and therefore the action is sustained e.g. coated granules.

CONTROLLED RELEASE DRUG DELIVERY SYSTEM

Controlled release drug delivery drug delivery system has a meaning that goes beyond the scope of sustained drug action it implies a predictability and reproducibility in the release kinetics. The release of drug from a controlled release drug delivery system proceeds at a rate profile that is not only predictable kinetically but also reproducible from one unit to another.

The improvement in drug therapy is represented by several potential advantages of controlled release system.

1. Avoid repeated administration and minimize the patient compliance problems
2. Minimize drug accumulation with chronic dosing.
3. Minimize local and systemic side effects.
4. Improve efficiency in treatment by
    a. Improving the bioavailability of some drugs.
    b. Reducing the fluctuation in drug concentration in blood level.
5. Economical
    a. Average cost of treatment over an extended period of time is less.
    b. Decrease in nursing time/hospitalization.

The following drugs are not suitable for formulating a sustained release medication.

- .Drugs with long biological half-life (more than 40 hours) e.g. digitoxin.
- Drugs with narrow requirement of absorption.

- Drugs with very short biological half-life (less than 1 hour) e.g. penicillin.
- Drugs which are very insoluble and whose availability is controlled by dissolution e.g. griseofulvin.

Controlled release drug system can be classified into four categories

1. Rate pre-programmed drug delivery system e.g. Implants.
2. Activation - modulated drug delivery system e.g. osmotic pump.
3. Feedback – regulated drug delivery system e.g. glucose triggered insulin delivery system.
4. Site – targeting drug delivery system e.g. niosomes, liposomes.

1. Rate pre-programmed drug delivery system

In this system the release of drug molecules from the dosage form has been pre-programmed at specific rate profiles. This was achieved by system designing which controls the molecular diffusion of drug molecules in and/or across the barrier medium within or surrounding the delivery system. Examples: Implants, Transdermal system.

2. Activation modulated drug delivery system

The release of drug molecule from this delivery system is activated by some physical, chemical, biochemical process and/or activated by the energy supplied externally. The rate of drug release is then controlled by regulating the process applied or energy input.

Based on the nature of the process applied or type of energy used, these activation modulated drug delivery system can be classified into three categories.

1. Physical: e.g. osmotic pressure activated drug delivery system – osmotic pump, iontophoresis activated drug delivery system.
2. Chemical: e.g. pH activated drug delivery system.
3. Biochemical: e.g. enzyme activated drug delivery system.

3. Feedback-regulated drug delivery system

The release of drug molecule from the delivery system is activated by a triggering agent, such as biochemical substance in the body and regulated by its concentration via some feedback mechanism.

e.g. bio responsive drug delivery system and glucose triggered Insulin delivery system.

4. Site targeting drug delivery system

In this system the drug molecule are circumventing the other tissues and moving towards the specific diseased site and get released. This will enhance the therapeutic effectiveness and reduces the toxicity to other healthy tissues and improve the treatment spectrum e.g. niosomes, liposomes and microspheres.

Niosomes and liposomes are unilamellar or multilamellar vesicles wherein aqueous solution is enclosed in highly ordered bilayer. They are capable of entrapping hydrophillic and hydrophobic drug molecules either in the aqueous layer or in lipid materials. They are osmotically active, stable, biodegradable, biocompatible and non-immunogenic.

MERITS OF NOVEL DRUG DELIVERY SYSTEM

1. Improved treatment of many chronic illness when symptom breaks through occurs when the plasma level of drug drops below the minimum effective level e.g. patients suffering from bronchial asthma and arthritis.
2. Increased bioavailability
3. Reduction in the incidence and severity of onto wards systemic side effects related to high peak plasma drug concentration.
4. Reduction in the total amount of drug administration over the period of drug treatment, this contributes to reduced incidence of systemic and local side effects.
5. Protection from first pass metabolism and gastrointestinal tract degradation.
6. Improved patient compliance resulting from the reduction in the number and frequency of doses required to maintain the desired therapeutic responses.
7. Targeting the drug molecules towards the tissue or organ reduces the toxicity to normal tissues.
8. Biocompatibility.
9. Versatile and pH dependent system release the drug whenever the body demands.
10. Maintenance of therapeutic action of a drug during overnight
11. Economic savings are claimed to be made from better disease management achieved with this system.

LIMITATIONS OF NOVEL DRUG DELIVERY SYSTEM

Through there are so many advantages in this system there are few factors that limits the usage. The following are some of the disadvantages

1. Variable physiological factors such as gastrointestinal pH, enzymes, activities, gastric and intestinal factors transit rates, find the severity of patient's disease which influences drug bioavailability from conventional dosage forms. All he above factors may also interfere with the absorption of drug from the system.

2. Drug having biological half-life of 1 hour or less are difficult to formulate or sustained release formulations. The high rate of elimination of such drugs from the body needs an extremely large maintenance dose which provides 8 to 12 hours of continuous therapy.

3. If it is once administered it may be difficult to stop the therapy for reasons of toxicity or any other.

4. It may unwise to include potent drugs in such system.

5. The product which tends to remain intact may become lodged at some sites. If this occurs slow release of drug from the dosage form may produce a high localised concentration of drug which causes local irritation or some side effects.

6. These products normally contain a large amount of drug. There is a possibility of unsafe over dosage. If the product is improperly made.

NIOSOMES – A REVIEW

Niosomes are one of the novel drug delivery system. Niosomes are unilamillar vesicles where in an aqueous solution is enclosed in highly ordered bilayer system. Both hydrophilic and lipophilic drugs can be entrapped either in aqueous layer or in vesicular membrane made up of lipid material

Because of the presence of the non-ionic surfactant and the lipid in niosomal formulation, drug molecule entrapped in niosomal drug molecule entrapped in niosomal drug delivery system is released slowly and the duration of action is prolonged. This concept is very useful for targeting drug for the treatment of cancer, parasitic and viral diseases. Niosomes have similar physical properties as liposomes and also behave as liposomes in vivo.

In the early 1960, Bangham et al. described liposomes, the phospholipid carrier as the drug carriers. Liposomes are prepared from phospholipids and require special conditions for handling and storage as they are liable to oxidation. Liposomes exhibits chemical instability and its physical properties vary with surface charge of membrane which also makes it relatively toxic

Concept of niosomes as carrier to deliver drugs to target organs and modify drug deposition may be similar to that of liposomes which suffer from certain disadvantages. The following are some the disadvantages of liposomes.

- Liposomes formulations are expensive.
- Liposomes are chemically unstable because of their predisposition to oxidative degradation.

- Purity of the natural phospholipids is another criterion that may affect the release of release of drug from liposomal drug delivery system.

Alternatives to phospholipids are thus of interest from technical view point and could also allow a wider study of the influence of chemical composition on the biological fate of vesicles.

Many synthetic amphiphiles form vesicles but as most of them are ionic and relatively toxic they are generally unsuitable for use as drug carriers. Niosomes are vesicles mainly consisting of non-ionic surfactants and lipid like cholesterol.

One of the reasons for preparing niosomes is the assumed higher chemical stability of surfactants than that of phospholipids, which are used in the preparation of liposomes. Due to the presence of ester bonds, phospholipids are easily hydrolysed. This can lead to phosphoryl migration at low pH.

Another type of degradation in liposomes is peroxidation of unsaturated phospholipids. In order to avoid peroxidation process, vesicles are often stored under nitrogen atmosphere. Unreliable reproducibility arising from use of lecithins in liposomes leads to additional problems and has lead scientists to search vesicles prepared from other material, such as non-ionic surfactants.

SALIENT FEARTURES

1. Niosomes entrap solute in a manner analogous to liposomes.
2. Niosomes are osomotically active and stable as well as they increase the stability of the entrapped drug.

3. Niosomes are more stable then the liposomes, because the phospholipids present in liposomes gets easily oxidised.

4. Handling and storage of surfactants require no special condition.

5. Niosomes possess an infrastructure consisting of hydrophobic and hydrophilic moieties together and as a result can accommodate drug molecules with a wide range of solubilities.

6. Niosomes improves oral bioavailability of poorly absorbed drugs and enhance skin penetration of drugs.

7. Niosomes exhibit flexibility in their structural characteristics (composition, fluidity, size) and can be designed according to the desired situation.

8. They allow their surface for attachment of hydrophilic group and can incorporate hydrophilic moieties in bilayer to bring about changes in the in-vivo behaviour of niosomes.

9. Niosomes dispersion in aqueous phase can be emulsified in non-aqueous phase to regulate delivery rate of drug for eternal application.

10. Niosomal surfactants are biodegradable, biocompatible and non-immunogenic.

11. Niosomes improve the therapeutic performance of the drug molecules by delayed clearance from the circulation and also it protects the drug from biological environment.

12. Niosomes are capable of encapsulating large quantities of material in relatively smaller volumes of vesicles.

NIOSOMAL DRUG DELIVERY

In recent years niosomes received great attention as potential drug delivery systems for different routes of administration parentally as well as orally. A tremendous work have been done to formulate drugs in niosomes which serve as carries for delivery of drugs, antigens, hormones and other bioactive agents.

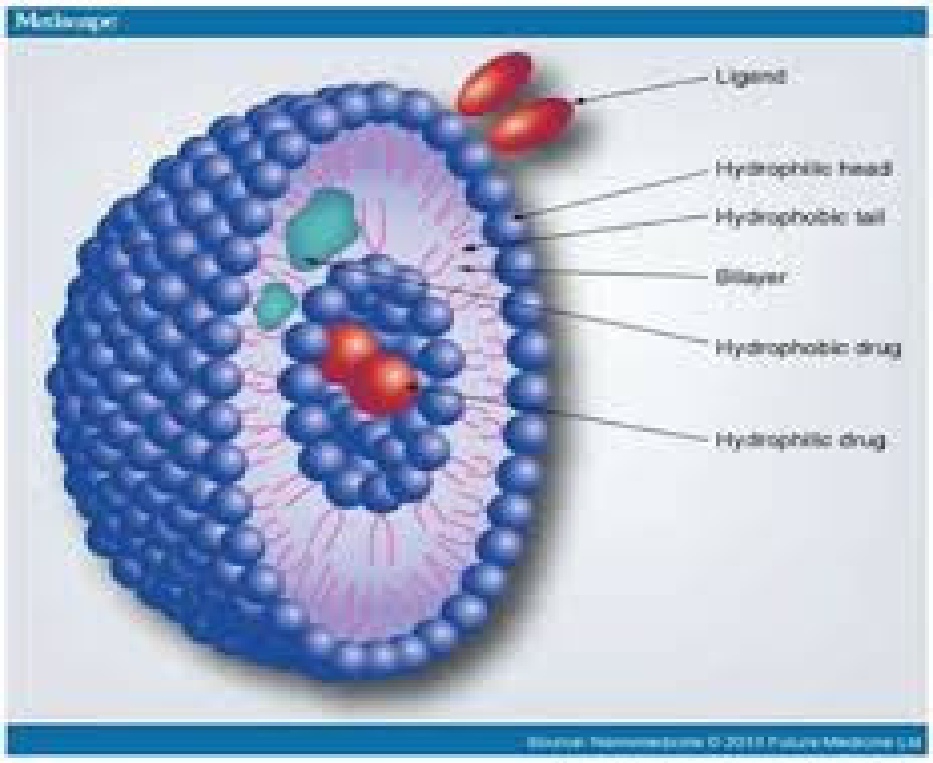
Niosomes are unilamellar or multilamellar vesicles wherein aqueous solution is enclosed in highly ordered bilayer structures made up of non-ionic surfactant with or without cholesterol and dicetyl phosphate and exhibit non-toxic, biodegradable, non-immunogenic and of suitable size and shape to accommodate a variety of substances.

Niosomes are formed by mixing phospholipids, non-ionic surfactant with aqueous medium. They are microscopic structures consisting of one or more concentric lipid bilayers surrounding aqueous compartments. A schematic diagram of a niosome, formed with non-ionic surfactant and cholesterol is shown in figure1

The surfactant and cholesterol form a variety of aggregates from micelles to large vesicles, which can be used as vehicles for drug delivery.

This is in accordance with the observation that a cone shaped and wedge shaped non-ionic surfactant and cholesterol to form bilayer membranes. These include carriers in oncology for delivery of anti parasitic agent, cosmetic formulations and as topical vesicles.

Figure 1.



Niosomes improves the therapeutic performance of the drug molecules by delayed clearance from the circular, protection the drug from biological environment. Niosomal formulations have been extensively studied to enhance the efficiency of the delivered drug via several routes of administration. In the last few years, many studies have also reported that topical delivery of niosomally encapsulated drugs may offer several advantages over conventional formulation such as decreased side effects and the control of the rate and extent of drug release into the skin layers.

Niosomes as a carrier for controlled drug delivery

Niosomes, the non ionic surfactant vesicles reported recently can entrap both hydrophilic drugs, either in aqueous layer or in vesicular membrane made up of lipid materials. It is reported to attain better stability than liposomes. It can prolong the

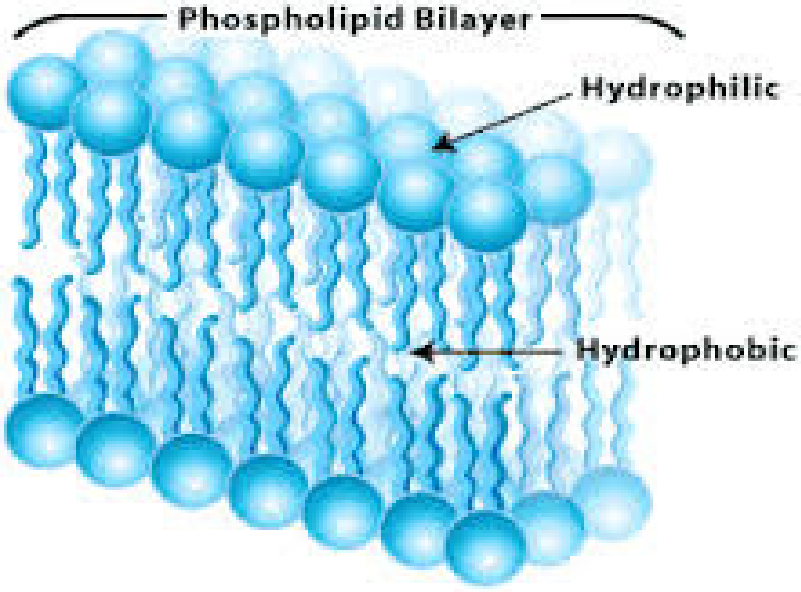
circulation of the entrapped drugs. Because of the presence of non-ionic surfactant wiped the lipid, there is better targeting of drugs to turnover, liver and brain. It may prove very useful for targeting the drugs for targeting cancer parasitic, viral and other microbial diseases more effectively.

Sources of niosomes

Glycerol containing phospholipids are the most commonly used component of niosomes formulation and represent more than 50% of the weight of lipids present in biological membranes. The most common phospholipids isphosphatidyl choline. It consists of a glycerol bridge linked to a pair of hydrophobic acyl hydrocarbon chains with a hydrophilic polar head group. phosphatidyl choline also known as lecithin can be derived from natural source. They are readily extracted from egg yolk and soyabean but less readily from bovine heart and spinal cord. The phospholipids are the major constituent of many cell membranes. They are often used as the principal phospholipids in noisome for a wide range of application both because of low cost relative to other phospholipids, the natural chargeand chemical inertness. The other phospholipids such as phosphatidyl serine, phosphatidyl ethanolamine, phosphatidyl glycerol, phosphatidyl inositol, phosphatidic acid and synthetic phospholipids such as dipalmitoylphosphatidyl choline, distearylphosphatidylcholine, dipalmitoyl serine and cholesterol were also used in the preparation of niosomes. Some of the structure of phospholipids is shown in figgure2

Niosomes have been prepared from several classes of nonionic surfactants e.g. polyglycerol alkyl ether, Glucosyldialkyl ether, crown ether, polyoxyethylene alkyl ether, ester linked surfactants, Brij and a series of spans and tweens

Figure 2



NIOSOMES FORMATION

Lipids capable of forming niosomes exhibit a dual chemical nature. Their head groups are hydrophilic and their fatty acyl chains are hydrophybic. It has been estimated that each zwitter ionic head groups of phosphatid33lycholine has bound on the order of 15 molecules of water, which explains its preference for their water phase. The hydrocarbon fatty acid chains on the other hand, vastly prefer each other company to that of water. This phenomenon can be understood in quantitative terms by considering the critical micelle concentration of phosphatidyl choline in water.

The critical micelle concentration is defined as the concentration of the lipid in water above which lipid forms micelles or bilayer structure rather than remaining in solutions as monomers. The large free energy charge between water and a hydrophobic environment explains the overwhelming preference of typical to

assemble in bilayer structure excluding water as much as possible as much from the hydrophobic core in order to achieve the lowest free energy level and hence the highest stability for the aggregate structure.

It is clear from the thermodynamic consideration that bilayer structure do not exist as such in the absence of water because it provides the driving force for lipid configuration.

TYPES OF NIOSOMES

Generally, the niosomes have been classified as a function of the number of bilayer (e.g. MLVs, SULVs) or as a function of size (e.g. LULVs, SULVs) or a function of the method of preparation (e.g. REV – Reverse Phase Evaporation, DRV – Dried Reconstituted vesicles).

1. Multilamellar Vesicles (MLVs)- 1000 to 5000nm
2. Larger Unilamellar Vesicles (LULVs)-100 to 1000nm
3. Small Unilamellar Vesicles(SULVs)-25 to 500 nm

- **Multilamellar vesicles**

It consists of number of bilayer surrounding the aqueous compartment separately. MLVs are the most widely studied type of niosomes. It is simple to make and they are mechanically stable upon storage for longer period of time. These vesicle are highly suited as drug carrier for lipophilic compounds.

- **Larger Unilamellar Vesicles**

Niosomes of this type have a high aqueous lipid compartment ratio. So the larger volume of bioactive material can be entrapped with a very economical use of

membrane lipids. These provides a number of advantages as compared to MLVs, including high encapsulation of water soluble drugs, economy of lipid and reproducible drug release rates. Because of the larger size of the vesicles high percentage of capture can be achieved.

The term large unilamellar usually means any structure larger than 100nm. A number of techniques for producing large unilamellar vesicles have been used including freeze – then cycling reverse phase evaporation method, ether injection method and calcium induced fusion method.

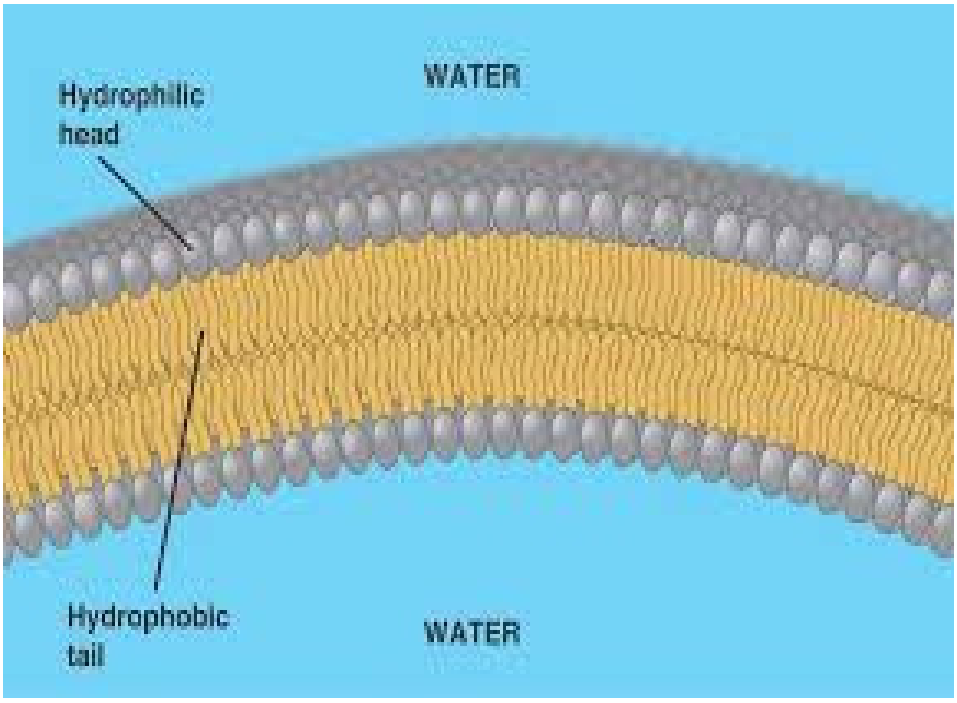
- **Small unilamellar vescicles**

These small unilamellar vesicles are mostly prepared from Multilamellar vesicles by sonication method, French press extrusion method or by homogenization method. They are thermodynamically unstable and are susceptible to aggregation and fusion. Their entrapped volume is small and percentage entrapment of aqueous solute is correspondingly low. The approximate size of small unilamellar vesicles are 25 to 500 nm in diameter.

PHYSICAL STATE OF VESICLES

Depending on the temperature, the bilayers of the vesicles are either in the liquid state or in gel state, the structure of bilayer is arranged in a well-ordered manner and in the liquid state; the structure of the bilayer is more disordered as shown in figure3.

Figure 3.



METHOD OF PREPARATION OF NIOSOMES

It depends both on the methodology used for their production and composition. Whatever the method, the basic reaction is same, that is hydration by the aqueous phase of the liquid phase either by surfactant or mixture of surfactant and cholesterol.

METHODS OF PREPARATION OF NIOSOMES

- **Multi Lamellar Vesicles (MLVs)**

    1. Thin film hydration method

- **Large Unilamellar Vesicles (LULVs)**

    1. Reverse Phase Evaporation method
    2. Calcium induced method

3. Dehydration / Rehydration of small Unilamellar Vesicles

4. Detergent removal method

- **Small Unilamellar Vesicles (SULVs)**

1. Sonication method

2. French press method

3. Ethanol injection method

4. Ether injection method

5. Homogenization

6. Dried Reconstituted Vesicles

I. Multilamellar vesicles

Bangosomes popularly known as MLVs are prepared according to the method of Bangham et.al. Lipids are dissolved in an organic solvent in a round bottom flast. A thin lipid layer is formed on the inside wall of the flask by removal of the organic solvent by rotary evaporation at reduced pressure.

Multilamellar vesicles were formed spontaneously when an excess volume of aqueous buffer was added to this dry lipid layer. Shaking (by hand or vortex mixer) yields a dispersion of multilamellar vesicles. Duration and intensity of shaking, the presence of charge inducing agent in the bilayer, ionic strength of the aqueous medium and lipid concentration is important parameters influencing the size and the encapsulating efficiency of multilamellar vesicles. The lipids formed were heterogeneous both in size and number of lamella.

II. Large unilamellar vesicles

Large unilamellar vesicles provides a number of important advantages as compared to multilamellar vesicles including high encapsulation of water soluble drugs, economy of lipid and reproducible drug release rates. However large unilamellar vesicles are perhaps the most difficult types of niosome to produce.

1. **Reverse phase evaporation method**

LULVs can be prepared by forming water in oil emulsion of phospholipids and buffer in the excess organic phase followed by the removal of organic phase under reduced pressure. The two phases are emulsified by sonication. The organic solvent is removed under vacuum and causes the phospholipid droplets to cool and form a viscous gel. These droplets of phospholipids merge with adjacent collapsed vesicles to form LULVs. The aqueous content of the collapsed droplet provides the medium required for suspension of newly formed niosomes. After conversion of gel to a homogeneous free flowing fluid, the suspension is dialyzed in order to remove the last traces of solvent. This method has gained widespread use for applications that require high encapsulation of water soluble drug. Entrapment efficiency up to 85% can be obtained with this method.

2. **Calcium induced method**

This method is used to produce unilamellar vesicles and it is of high interest for the present investigation. This method takes advantages of the fact that small vesicles aggregate in the presence of calcium and subsequently fuse.

In calcium induced method, the percentage of encapsulation depends on the concentration of lipid. In this method only 30% of encapsulation of the drug is

expected. The vesicles obtained will be in the size range of 200 to 1000 nm in diameter. Large niosomes could be produced on addition of EDTA.

The flocculent precipitate is obtained as a result of aggregation of the negatively charged vesicles by calciumcations. After incubation, the membranes fuse to give extended sheet of phospholipids lamella, to roll up with calcium ions as a driving force. On addition of EDTA, this lamella is released and subsequently formed LULVs. This technique has the advantage that it does not expose lipids or entrapped material to deleterious chemicals or physical conditions.

3. Dehydration / rehydration of small unilamellar vesicles

In this method, the sonicated vesicles are mixed in an aqueous solution with the solute to be encapsulated and the mixture is dried under a stream of nitrogen. As the sample is dehydrated, the small vesicles fused to form a multilamellar film that effectively sandwitches the solute molecules between layers. Upon rehydration large vesicles are produced which have encapsulated a significant proportion of solute. The optimal mass ratio of lipid to solute was approximately 1:2 to 1:3. This method has potential application to large scale production. Since it depends only on controlled drying and rehydration processes and does not require extensive use of organic solvents, detergents or dialysis system.

4. Detergent removal method

Removal of detergent from mixed micelles formed by solubilizationof dried lipid mixtures or formed niosomes with detergent containing aqueous phase result in the formation of unilamellar vesicles. This is gentle method where no strong mechanical force and no high temperature are applied. The preparation procedure

should include a step to minimise residual detergent level after niosomes formation. The techniques reported for the removal of detergents includes dialysis and column chromatography.

III. Small unilamellar vesicles

1. Sonication Method

Method for the preparation of sonicated small unilamellar vesicles have been reviewed by bangham and horne. The usual MLVs and SULVs formed are sonicated either with a bath type sonicator or a probe sonicator, under an inert atmosphere (usually nitrogen or argon) to get the small unilamellar vesicles. During sonication the multi lamellar vesicle structure is broken down and unilamellar vesicles with high radius of curvatures are formed.

2. French press method

Dispersion of MLVs can be converted to small unilamellar vesicles by passage through a small orifice under high pressure. A French pressure cell was used by Hamilton and guo for this purpose. MLV dispersion is placed and extruded at about 20,000 Psi at 4^0 C. A heterogenous population of vesicles ranging from several micrometers in diameter to SULVs size was formed. These niosomes are more stable than sonicated ones and can be used advantageously as drug delivery carriers.

3. Ethanol injection method

An alternative method for producing small niosomes that avoids both sonication and high pressure is the ethanol injection method, first described by batzriand koru. Lipids dissolve in ethanol are rapidly injected into an axcess of buffer solution or other aqueous medium through a fine needle. The force of the injection is

sufficient to achieve complete mixing, so that the ethanol is diluted in water and phospholipid molecules are dispersed evenly throughout the medium. This procedure can yield a high proportion of SULVs.

4. Ether injection method

This method is similar in concept to ethanol injection method. This is introduced by Deamer and Bangham in 1976. This method provides a means of making small lamellar vesicles by slowly introducing a solution of lipid dissolved in diethyl ether into warm aquous medium. The lipid mixture is injected into aaqueous solution of the material to be encapsulated (using a syringe type infusion pump) at 55^0 – 65^0 C or under reduced pressure. Subsequently the residual ether was removed under vacuum and it leads to the formation of single layer vesicles.

5. Homogenization

Homogenization of MLVs or other lipid dispersions by high sheer homogenizers like the micro fluidizer produces unilamellar vesicles. The SULVs formed are longer than the minimal size formed by sonication and significant amounts of larger particles are also presents. The size of the vesicles produced by micro fluidizer depends on the pressure used and on the number of passes of the preparation through this device and on the niosomal lipid composition.

6. Dried Reconstituted Vesicles

This method has been sought to disperse the solid lipid into a finely divided form before contact with the aqueous fluid, which will form the medium for the final suspension. Freeze drying was used instead of drying the lipid and the suspension of SULVs are frozen and lyophilized. The dried SULVs are very highly organized into

membrane structures which on addition of water can be rehydrated, fused and resealed to form vesicles with high capture efficiency.

METHODS OF CONTROLLING NIOSOMES SIZE

The vesicles size can have dramatic effect on the in vivo behaviours of niosomes. Therefore their size will have to be controlled with in reasonable and verifiable limits. Three possible approaches have been explored for achieving particle size distributions of niosomes.

a) Fractionation.

b) Homogenization

c) Extrusion

a. Fractionation

Two method have been popular for fractionating defined sized niosomes from a heterogeneous population namely centrifugation and size extrusion chromatography. Both can be used to enrich the products with the desire particles size but are limited in terms of the volume that can be easily handled.

i. Centrifugation

Niosomes Sediment in a centrifugal field at a rate is related to size and density. Large niosomes composed of neutral lipids such as phosphatidyl choline can easily be pelleted at fairly low gravitational forces in centrifuge under proper conditions. The smaller niosomes will remain in the supernatant

ii. size extrusion chromatography

Column chromatography has been used for many years as an analytical method to measure the particle size of niosomes.

Preparative scale chromatography has also been applied to isolate niosomes of fairly homogenous sizes. This method is useful for separating small unilamellar vesicles from large structures.

Typically a column of sepharose48 is used. As the column is washed with buffer, larger niosomes elute in the void volume, while SULVs appear with the included volume. Larger pore size chromatographic media have been used in similar fashion to fractionate populations of larger particles.

b. Homogenization

When fairly small particles are desirable, homogenization has proven to be a useful approach. The average particles size of niosomes dispersion can be reduced by passage under high pressure through a homogenizer. Microfluidizer was to generate vesicles in the size range of 50 – 200 nm. Conventional homogenizer and high sheer mixers are also used for downsizing niosomes.

c. Extrusion

i. capillary Pore Membrane Extrusion

A technique that is used for the production of defined size and narrow size diatributiion is based on the extrusion of heterogenous membranes under pressure. Polycarbonate membranes have pores of defined size normally do not bind niosomes. This simple technique can reduce a heterogenous population of MLVs and reverse

phase evaporator vesicles to a much more homogenous suspension of vesicles exhibiting a much more homogenous suspension of vesicles exhibiting a mean particle size that approaches the diameter of the pores through which they are ectruded.

ii. Ceramic Extrusion

If the size of niosomes is greater than the membrane pore diameter pore diameter the pores of membranes tends to clog. The clogged membranes cannot pore of the membrane used to clog. The clogged membranes cannot easily be cleared because the filter housing configuration does not allow back-flushing. To overcome this limitation, ceramic membrane was used. Extrusion of unsized niosomes through asymmetric ceramic filter yields sied niosomes having a selected average size between 0.1 and 0.4 micrometer. The suspension may be alternately passes through the membrane is an outside to inside direction to maintain the membrane in an unclogged condition. The average size of niosomes may further be reduced by passage through similar types of ceramic filters that have been rated at smaller inner surface pore diameter.

CHARACTERIZATION OF NIOSOMES

1. Vesicle Diameter

Niosomes similar to liposomes assume spherical shape and its diameter can be determined by using light microscope, photon correlation microscopy and freeze-fracture electron microscopy. The shape of vesicle can be confirmed by transmission electron microscopy.

2. Entrapment Efficiency

After preparing niosomal dispersion, unentraped drug is separated by dialysis, centrifugation or gel chromatography. The drug remaining entrapped in niosomes is determined by complete vesicular disruption using 50% n-propanol or niosomes is expressed as,

$$\text{Entrapment efficiency} = \frac{\text{amount of drug entrapped}}{\text{total amount of drug added}} \times 100$$

Entrapment efficiency can also be determined by the difference between the drug used for formulation and the amount dialysed or obtained in supernatant. The intercalation of cholesterol in bilayers decreases the entrapment volume and thus entrapment efficiency decreases. The entrapment efficiency increases with increase in concentration and lipophilicity of surfactant.

3. Invitro release rate

Release of drug can be monitored by dialyzing dilute niosomal suspension against buffer at definite temperature and determining the drug content of dialysate. All the dialysis studies should be carried out in phosphate buffer (900ml, pH 7.4), maintained at 37.5°C at 100 r.p.m. The absorbance of the drug was measured.

Factors affecting vesicle size, entrapment efficiency and release characteristics

1. **Drug**

- Entrapment of drug in niosome increases the vesicle size probably by interaction of solute with surfactant head groups, increasing the charge and mutual repulsion of surfactant bilayer thereby increasing vesicle size. In

polyoxyethylene (PEG) coated vesicle some drugs are entrapped in long Peg chain thus reduce the tendency to increase the size.

- Degree of entrapment is affected by hydrophilic-lipophilic balance of a drug. For a series of tweens, Raja Naresh et.al reported maximum emtrapment of water soluble drug diclofenac sodium in hydrophilic surfactant tween 60 and Chandraprakash et.al reported maximum entrapment of slightly water soluble drug methotrexate in lipophilic methotrexate in lipophilic surfactant span 60

2. Amount and type of surfactant

- Mean size of niosomes increases regularly with increase in hydrophilic-lipophilicbalance (HLB) from span 85 (HLB 1.8) to span 20 (HLB 8.6) because surface free energy decreases on increasing hydrophobicity of surfactant.

- Linear correlation is between concentration of lipid and entrapment efficiency.

- Phase transition temperature (T_c) of surfactant also effects entrapment efficiency that is, surfactant having higher T_c provides the highest entrapment.

- Yoshioka et.al reported linear correlation between concentration of lipids and entrapment efficiency. Phase transition temperature T_c provides the highest entrapment.

3. Cholesterol content and charge

- Inclusion of cholesterol in niosomes increases its hydrodynamic diameter and entrapment efficiency.

- Presence of charge tends to increase the inter-lamellar distance between successive bilayers in Multilamellar vesicle structure and leads to grater overall entrapped volume

- Vesicle size is slightly decreased as charge might increase the membrane curvature.

- Presence of cholesterol in bilayer composition due to its membrane stabilizing activity reduces permeability and improves retention of solute.

- Baillie et.al reported that incorporation of 50%cholesterol in surfactant composition reduces vesicle permeability.

4. Method of preparation

Method of preparation of niosomes such as hand shaking, ether injection and sonication, developed on the basis of liposome production technique have been reviewed by Khandare et al. hand shaking method from vesicles with grater diameter (350 to 1300 nm) compared to those prepared by ether injection method (50 to 1000nm). Sonication of MLVs prepared by above methods, either with probe sonicator or bath type sonicator from unilamellar vesicles with considerably reduced diameter. Increase in sonication time results in concomitant reduction in vesicle diameter. Hydrating the lipids helps to reduce the size of vesicles prepared by hand shaking method.

Safford et al extruded the liposomal dispersion prepared by hand shaking method through 1000 nm. Small sized niosome can be produced by Reverse Phase Evaporation (REV) method. In this method emulsion of aqueous phase is organic solvent containing lipid is prepared by sonication, followed by evaporation of organic

solvent, resulting in the formation of vesicles. Micro fluidization method gives grater uniformity and smaller size vesicles. Parthasarathy et al prepared niosomes by trans membrane pHgradient drug uptake process (TmpH) or remote loading showed grater entrapment efficiency, better retention and slower release of drug

Niosomes bearing 5, 6 – carboxyfluorescein prepared by either injection method slowed entrapment efficiency significantly higher than those prepared by hand shaking method or sonication. Niosomes prepared by remote loading method showed grater entrapment efficiency and slower release of drug.

5. **Osmotic effect**

Addition of hypertonic salt solution to suspension of niosomes brings about reduction in vesicle diameter with concomitant water efflux which may be due to pumping out of vesicle content where as in hypotonic salt solution, there is initial slow release with slight swelling of vesicles probably due to inhibition of eluting fluid from vesicle, followed by faster release which may be due to mechanical loosening of vesicles under osmotic stress.

INVITRO BEHAVIOUR OF NIOSOMES

In vivo niosomes have been equiactive to liposomes in improving the therapeutic performance of drug and distribution in body follows the pattern of their colloidal drug delivery systems. Although liver, lungs spleen and bone marrow are responsible for disposition of a major part of niosomes yet their level in liver is always significantly higher due to the natural vectoring process. Variation in size also influences the disposal of niosomes from blood. Variation in size also influences the

disposal of niosomes from blood. Large sized may reside in lungs and small sized niosomes in liver and spleen and then enter systemic circulation

It appears that liposomes, niosomes are also taken up by liver and break down substantially to release the free drug which re-enters the circulation nd maintain the plasma drug level. Parthesarrathy et al found that niosomes are stable in plasma. However non ionic surfactants in higher concentration delipidize the low density lipoproteins.

Moser et al found that niosomes bearing haemoglobin are physically stable with plasma protein compartment. Albumin and transferrin were identified and determined to absorb on vesicle without destablizing them. Erythrocytes donate cholesterol to niosomes particularly to cholesterol-free and cholesterol poor niosomes maintain their integrity in body as well as keeping them less vulnerable to destabilization.

NIOSOMES INTERACTION WITH THE CELLS

Niosomes can interact with cells via five mechanisms. All mechanism are explained in brief as follows

1. Inter membrane transfer

Inter membrane transfer of lipid component can take place upon close approach of the two phospholipid bilayers without the disruption of niosome. Interaction can take place between niosomes and in certain circumstances proceed to the extent of destroying the niosomes altogether

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2. Contact release

Contact release of aqueous content of niosomes occurs in which contact with the cell membrane causes an increase in permeability of the niosomal membrane. This leads to release of water soluble solutes in high concentration in the close vicinity of cell membrane through which these solutes may pass. This phenomenon can provide very effective means for introducing material into specific cells without the need for ingestion of the whole niosome.

3. Adsorption

Adsorption of niosomes to the cells surface occurs with little or no internalization of either aqueous or lipid components. It may take place either as a result of physical attractive forces or lipid component. It may take place either as a result of physical attractive forces or as a result binding by specific receptor to the vesicle membrane.

4. Fusion

They may undergo fusion with plasma membrane of target cells. Close approach of niosomes and cell membrane can lead to fusion of the two resulting in complete mixing of niosomal contents into the cytoplasm.

5. Endocytosis

The niosome is engulfed by cell. The lysozyme present in the cytoplasm degrades or digests the membrane structure of niosomes there by resulting the entrapped material into the medium.

STABILITY OF NIOSOMES

Three types of stability studies can be carried out in drug encapsulation niosomes. They are:

- Physical stability
- Chemical stability
- Stability in biological fluids.

Physical stability

Niosomes can change their physical characteristics in several ways

1. Vesicle size may be changed because of aggregate formulation and fusion.
2. Phase separation of bilayer components may occur upon the storage
3. Leakage of encapsulated material from niosomes

1. Vesicle size changes upon storage of phosphatidyl choline containing niosomes over pharmaceutically relevant time intervals can be minimized by selection of charge inducing agents. Mostly negatively charged phospholipids (phosphatidyl glycerol) are used to stabilize the niosomes.

2. Phase separation can occurs when the bilayer composition changesbecause of chemical degradation reaction or when the bilayer goes through temperature cycles. Proper selection of bilayer component can be avoid these problems. Sometimes phase separation occurs invivo, when bilayer components are selectively drawn from the bilayer plasma components. If this effect is undesired, then more rigid bilayer plasma components must be prepared.

3. When niosomal preparation are stored at various temperatures, the permeability of bilayer is highly dependent on the physiochemical properties of the bilayer and drug.

Three categories of drugs can be discussed

- Highly hydrophilic, non-bilayer interacting drugs.
- Drug with some lipophilicity.
- Strongly lipophilic drugs.

In category first the presence of cholesterol in bilayer of the egg phosphatidyl choline niosomes dramatically reduces the permeability. Gel state bilayer permeability is low with or without cholesterol. Shelf life of the niosomes in aqueous media with proper pH might easily meet the industrial demands. The second category tends to be difficult in keep entrap over period of months. As long as outside sink condition prevail, the third category of strongly lipophilic drugs has a high affinity of the bilayer and drugs stay over a long period of time, independently of the state of bilayer.

In presence of hydrolysis/oxidation reaction, products can affect bilayer properties although lysophosphatidyl choline is known as the lipid bilayer solubilizer. The solubilizing effect of lysophosphatidyl choline in niosomes is neutralized by the presence of fatty acid in the bilayer.

Stability of niosomes stored in freeze dried form:

The different types of cryoprotectant and the possible mechanism of action have been discussed by Crow et al and Ozer et al for niosomes stabilization. Usually

sugar are used as cryoprotectant. Some other types of excipients also have been reported to exert cycloprotective effect. A number of effects may contribute to cycloprotective action

1. The formation of amorphous glass structures during the freeze drying process might avoid mechanical damage inflicted by the crystals. It is recommended to store these cakes below glass transition temperature.

2. The sugars may interact with polar head groups of the phospholipids and stabilize the membrane when the bilayer stabilizing water is removed by sublimation.

The proliposome concept

In proliposome formulations, liposomes are formed by hydrating lipids at bed side. The dry lipids (formation of film on the sides of the sides of the glass vessel is the form of freeze dried cake) are hydrated by shaking with an aqueous medium just before injection.

Another way to improve stability of lipids is by covalently cross linking the membrane using methods such as gluteraldehyde fixation or polymerization. The membrane stability is increased by imparting charge to the niosomes.

Chemical stability

The stability of niosomal formulations depend on the chemical nature and stability of lipid components. The main bilayer component of niosomes contains drug and phospholipids. Hydrolysis and peroxidation are the two degradation processes which affects the stability of niosomal formulations. Different analytical techniques are available to monitor hydrolysis and oxidation.

Lipid hydrolysis

Grid et al described in number of articles, different variables influence the hydrolysis reaction of phosphatidyl choline. The major phospholipids in the niosome preparations are the charge inducing phospholipids like phosphatidyl glycerol. Apart from pH, other experimental conditions like temperature, ionic strength and ultra sonifications were reported to influence hydrolysis reaction. Many investigations choose the formation of lysophosphatidyl choline as a standard measure for the chemical stability of phospholipids. Since the presence of lysophosphatidyl choline in lipid bilayer enhances the permeability of niosomes. This problem can be minimized by using proper source of phospholipids.

Most of phospholipid niosomal dispersions contain unsaturated acyl chains as a part of their molecular structures. These chains are vulnerable to oxidative dehydration (lipid peroxidation). This peroxidation can occur during preparation and storage. Peroxidation of phospholipids produces the formation of cyclic peroxides and hydroperoxides and can be minimized by a number of ways.

- Minimize use of unsaturated phospholipids.
- Use of nitrogen or argon to minimize the exposure to oxygen.
- Use of light resistance containers.
- Removal of heavy metals (EDTA).
- Use of antioxidants like tocopherol or butylatedhydroxy toluene (BHT).

Stability of niosomes in biological fluids

Stability of entrapped drug in niosomes in blood plasma have been studied for a decade. The instability of niosomes in plasma appears to be the results of the transfer of bilayer lipids to albumin and high density lipoproteins. The presence of cholesterol prevents this instability.

Generally niosomes are stable structures; Jain did not observe any gross morphological changes on storage for three months. Yoshioka and Florence found them stable in even emulsified form. Baaillie et al determined the stability in butter and suggested that a substantial amount of entrapped solute would be retained under long term storage conditions.

Toxicity of niosomes

Non-ionic surfactants used in niosomes are non-toxic and no toxic effects have been reported so far in animal studies due to the use of niosomes as drug carrier. Rogerson et al. in their experiment on 70 male NMRI mice, did not report any fatalities that could be attributed to the preparation. The toxic side effects directly related to drugs are also reduced.

Applications

The niosomes has a wide range of applications.

1. The wide applicability is in the diagnosis and therapy of cancer.

2. Most of the anti-tumour drugs are highly toxic to normal cells and have a narrow therapeutic range. The niosomes encapsulated anti-tumour drugs increases the shelf life of the drug and reduces the toxicity.

3. The niosomes are also used in the enzyme replacement therapy.

4. In cosmetics, niosomes containing vitamin c prepration and sunscreen preprations are available. Recently sustained release niosomes containing insect and perfume loaded niosomes containing insect repellent and perfume loaded niosomes have been reported.

5. In the agricultural field it has been attempted to prepare pesticides containing niosomes for sustained release and also introduce the genetic materials into the plant and bacterial cell using the niosomes.

6. Niosomes can also have the applications in Physical Science.Niosomes have been used as the means of maintaining charge sepration in the photo catalytic cleavage of water.

7. The use of niosomes for the delivery of food ingredients of offers the totally new technology with unique delivery opportunities.

8. Improvement of flavour of cheese, targeted delivery of fuctional food ingredients, the synergistic delivery of ascorbic acid and for enhancing the antioxidant activity and the stabilization of vitamins in foods.

Niosomal drug delivery is potentially applicable to many pharmacological agents for their action against diseases. Some of its therapeutic applications are:

1. **Targeting of bioactive agents**

    a. **To Reticulo Endothelial System:**

The vesicles are preferentially taken up by the cells of reticulo endothelial system. The uptake of niosomes by the cells is also by circulating serum factors known as

opsonins which mark them for clearence. Suchlocalized drug accumulation has been exploited in treatement of animal tumors known to metastasize to the liver and spleen and in parasitic infestation of the liver.

b. To Organs Other Than Reticulo Endothelial System:

The carrier systems can be directed to specific sites in the body by the use of antibodies. Immunoglobulin seems to bind quite readily to the lipid surface thus offering a convenient means for targeting of carrier. Many cells posses the intrinstic ability to recognize and bind particular carbohydrate determinants and this can be exploited to direct carrier systems to particular cells.

2. Neoplasia

Doxorubicin, the anthracycline antibiotic with broad spectrum antitumor activity, shows a dose dependent irreversible cardio toxic effect. Niosomal delivery of this drug to mice bearing s-180 tumour increased their life span and decrease the rate of proliferation of sarcoma.

Niosomal entrapment increased the half-life of the drug, prolonged its circulation and altered its metabolism. Intravenous administration of methotrexate entrapped in niosomes to s-180 tumour bearing mice resulted in total regression of tumor and also higher plasma levels and slower elimination.

3. Leishmaniasis

Niosomes can be used for targeting of drugs in the treatment of diseases in which the infecting organism resides in the organs of reticulo endothelial system. Leishmaniasis is such a diseases in which parasites invades cells of liver and spleen. The commonly prescribed drugs are antimonial which are related to arsenic and at

high concentration ; they damage the heart, liver and kidney but encapsulating such drugs in niosomes targets the drug to the infected organs and greatly reduces the dose needed to treat the infection and also reduces the toxicity.

Baillie et al reported increased drug efficacy of niosomal formulation and that the effect of two doses given on successive days were adequate. The study of antimony distribution in mice, performed by hunter et al, showed higher levels after intravenous administration of the carrier forms of the drug. Carter et al concluded that only multiple dosing with drug loaded sonicated vesicles were effective against parasites in liver, spleen and bone marrow while other treatments reduced parasites burdensin liver but either failed to effect spleen and bone marrow or were effective but toxic.

4. Delivery of peptide drugs

Yoshioka et al investigated oral delivery of 9-desglycinamide, 8-arginine,and vasopressin entrapped in niosomes and the in vitro intestinal loop model reported that stability of peptide increased significantly.

5. Immunological application of niosomes

Niosomes have been used for studying the nature of the immune response provoked by antigens. Brewer and Alexander has reported niosomes as potent adjuvants for protein antigens. Niosomes offer many advantages over other adjuvants in terms of immunological selectivity, low toxicity and stability.

6. Niosomes as carrier for haemoglobin

Niosomes can be used as a carrier for haemoglobin. Niosomes suspension shows visible spectra super imposable onto that of free haemoglobin. Vesicles are

permeable to oxygen and the haemoglobin dissociation curve can be modified similarly to non-encapsulated haemoglobin.

7. Transdermal delivery of drugs by niosomes

Slow penetration of drugs through skin is the major drawback of transdermal route of delivery. An increase in the penetration rate has been achieved by transdermal delivery of drug incorporated in niosomes.

8. Niosomes in the treatment of neoplastic diseases

I. Niosomes in the treatment of infectious diseases

Application of niosomes as a drug carrier for anti- infectious drugs might be an effective therapy by increasing the selectivity of delivery and decreasing the toxicity of applied drugs.

II. Niosomes encapsulated anti protozal Drugs

Anti-malarial drugs - e.g. Primaquine

Anti-fungalagents - e.g. Ampnotericin-B, Griseofulvin.

Anti leishmanial agents – e.g. 5-fluoro cytosine

III. Niosomes encapsulated anti-bacterial drugs

E.g. Streptomycin, Gentamycin, Amoxycillin,Rifampicin,Dapsone etc.

IV. Niosomes as carriers of antigens

E.g. Diptheria toxoid,Tetanus toxoid, Rabies glycoprotein.

V. Intra pulmonary delivery

E.g. Metaproterenol, Terbutaline, Sodium cromoglycate

VI. Intra peritoneal administration

E.g. Cisplatin, Cytosine, Bleomycin

VII. Intra muscular administration

E.g. Insulin

VIII. Topical route of administration

E.g. Glucocorticoids, Indomethacin, Vitamin D3

IX. Intra ocular delivery

E.g. Gentamycin, Amphotericin-B,Triamcinolone acetonide

Other applications

i. Sustained Release

Drugs that are rapidly excreted or metabolized possess "Sawtooth" drug kinetics. This is undesirable especially for agents which have low therapeutic index. Experiments have suggested that niosomes can provide relatively constant and sustained blood stream levels of drug concentration. Many anti-tumour drugs are cleared from the blood stream very quickly, while the same agents, entrapped in niosomes, persist in the blood for hours. In contrast to other sustained release formulations, niosomes can be injected into circulation and thus serve as an intra vascular drug depot.

Liver acts as a depot for the drug after niosomes are taken up by the liver cells. Sustained release action of niosomes could be applied to drugs with low therapeutic

index and low water solubility. Since these could be maintained in the circulation via niosomal encapsulation.

i. Topical Action

Drug delivery through niosomes is one of the approaches to achieve localized drug action, since their size and low penetrability through epithelium and connective tissue keeps the drug localized at the site of administration.

Localization of drug action results in enhancement of efficacy or potency of the drug and at the same time reduces its systemic toxic effects. The leishmaniasis causing organism in an intra-cellular parasite residing primarily in endocytotic vacuoles in phagocytic monomolecular cells. This being the primary site of niosomes update in-vivo. Antimonial encapsulated within niosomes are taken up by mononuclear cells resulting in localization of the drug causing an increase in potency and hence decrease in dose and toxicity.

The evolution of niosomal drug delivery is still at an infancy stage, but this type of drug delivery system has shown promise in cancer chemotherapy and anti-leishmanial therapy. Niosomes may prove to be a convenient drug delivery system for other diseases. Niosomes are promising vehicle for drug delivery. It is less toxic and improves the therapeutic index of drugs by restricting its action to target cells.

ADVANTAGES OF NIOSOMES

1. Biocompatibility, the components of niosomes is formed in the body itself.
2. Polar and nonpolar bioactive materials of various molecular sizes can be incorporated in the noisome.

3. The entrapped compounds are protected from degradation because of the limited permeability of niosomes.

4. Targeting the drugs to liver, lungs and spleen are easier, macrophages of reticulo endothelial system take up niosomes immediately after systemic administration and direct them to liver and spleen.

5. Niosomes exhibit flexibility in their structural characteristics. So that it can be suited for the desired situations.

6. Niosomes can be administered by different routes (IV, intra peritoneal, oral and topical) and it can be used for a wide range of ailments.

7. Niosomes can enhance the skin penetration of drugs.

LITERATURE AND REVIEW

1. Mahmoud Mokhtar et al.,(2008) developed proniosomal gels or solutions of flurbiprofenbased on span 20 (Sp 20), span 40 (Sp 40),span 60 (Sp 60), and span 80 (Sp 80) without and with cholesterol.Nonionic surfactant vesicles (niosomes)formed immediately upon hydrating proniosomal formulae. The entrapment efficiency (EE%) of flurbiprofen(a poorly soluble drug) was either determined by exhaustive dialysis of freshly prepared niosomesor centrifugation of freeze-thawed vesicles. The influence of different processing and formulation variables such as surfactant chain length, cholesterol content, drug concentration, total lipid concentration, negatively or positively charging lipids, and the pH of the dispersion medium on flurbiprofen EE% was demonstrated. Also, the release of the prepared niosomes in phosphate buffer (pH 7.4) was illustrated. Results indicated that the EE% followed the trend Sp 60 (C18)>Sp 40 (C16)>Sp 20 (C12)>Sp 80 (C18). Cholesterol increased or decreased the EE% depending on either the type of the surfactant or its concentration within the formulae. The maximum loading efficiency was 94.61% when the hydrating medium was adjusted to pH 5.5. Increasing total lipid or drug concentration also increased the EE% of flurbiprofen into niosomes. However, incorporation of either dicetyl phosphate (DCP) which induces negative charge or stearyl amine (SA) which induces positive charge decreased the EE% of flurbiprofen into niosomal vesicles. Finally, in vitro release data for niosomes of Sp 40 and Sp 60 showed that the release profiles of flurbiprofen from niosomes of different cholesterol contents is an apparently biphasic release process. As a result, this study suggested the potential of proniosomes as stable precursors for the immediate preparation of niosomal carrier systems.

2. Kumar, Dhiraj et al (2011) described a simple, accurate, precise and cost effective UV-VIS Spectrophotometric method for the estimation of Bosentan Monohydrate, an anti-hypertensive drug, in bulk and pharmaceutical dosage form. The solvent used was Acetonitrile and water in ratio of 10:90 and the lmax or the absorption maxima of the drug was found to be 272nm. A linear response was observed in the range of 10- 100µg/ml with a regression coefficient of 0.999. The method was then validated for different parameters as per the ICH (International Conference for Harmonization) guidelines. This method can be used for the determination of Bosentan Monohydrate in quality control of formulation without interference of the excipients.

3. Gabriel J. Robbie et al., (2001) formulated 62.5 mg and 125 mg tablets for the long-term treatment of pulmonary arterial hypertension. In theoriginal NDA submission the sponsor proposed to use 1% sodium lauryl sulfate in water as the dissolution specification medium. However, the Office of Clinical Pharmacology and Biopharmaceutics suggested the use ofi `% sodium lauryl sulfate in water because of good (/0 dissolved in 30 minutes) dissolution. After a series of teleconference calls, the Sponsor was requested to provide data supporting the lack of "sink conditions" with '% sodium lauryl sulfate in water. The sponsor has therefore performed comparative dissolution of bosentan tablets in('% and 1% sodium lauryl sulfate in water and submitted the results in the present submission

4. A.Ashok Kumar et al., (2011) described a simple, accurate, precise, specific and highly sensitivemethod for the determination of bosentan present in pharmaceutical dosage forms. Themethod is validated for the determination of Bosentan in bulk and tablet dosage form.Bosentanis a dual endothelin receptor antagonist used in the treatment of pulmonary arteryhypertension (PAH). The solvent

used was methanol: water (60:40) and the λmax or theabsorption maxima of the drug was found to be 270nm. A linear response was observed inthe range of 10-90µg/ml with a regression coefficient of 0.9993. The method was thenvalidated for different parameters as per the ICH (International Conference forHarmonization) guidelines. This method can be used for the determination of Bosentan inquality control of formulation without interference of the excipients.

5. Ali Nasir et al., (2012) designed and developed novel drug delivery system (NDDS) having two prerequisites. First, itshould deliver the drug in accordance with a predetermined rate and second it should releasetherapeutically effective amount of drug at the site of action. Conventional dosage forms areunable to meet these requisites. Niosomes are essentially non-ionic surfactant-basedmultilamellar or unilamellar vesicles in which an aqueous solution of solute is entirely enclosedby a membrane resulting from the organisation of surfactant macromolecules as bilayer.Niosomes are formed on hydration of non-ionic surfactant film which eventually hydratesimbibing or encapsulating the hydrating aqueous solution. The proposed review deals withcomposition, methods of preparation, and applications of niosomes in the pharmaceutical field.The main aim of development of niosomes is to control the release of drug in a sustained way, Modification of distribution profile of drug and for targeting the drug to the specific body site.

6. Dhiraj Kumar et al., (2011) described a simple, accurate, precise and cost effective UV-VIS Spectrophotometric method for the estimation of Bosentan Monohydrate, an anti-hypertensive drug, in bulk and pharmaceutical dosage form. The solvent used was Acetonitrile and water in ratio of 10:90 and the lmax or the absorption maxima of the drug was found to be 272nm. A linear response was observed in the range of 10- 100µg/ml with a regression coefficient of 0.999. The

method was then validated for different parameters as per the ICH (International Conference for Harmonization) guidelines. This method can be used for the determination of Bosentan Monohydrate in quality control of formulation without interference of the excipients.

7. D.Akhilesh et al., (2012) approaches to stabilize niosomal drug delivery system without affecting its properties of merits have resulted in the development of the promising drug carrier "Proniosomes". Proniosomes is a dry formulation using suitable carrier coated with non-ionic surfactants and can be converted into niosomes immediately before use by hydration. These proniosomederived niosomes are as good as or even better than conventional niosomes.Glipizide loaded Sorbitol, Maltodextrin and Mannitol based proniosomes were prepared by slurry method with different surfactant to cholesterol ratio.Theproniosome formulations were evaluated for FT-IR study, angle of repose and scanning electron microscopy.The niosomal suspensions were further evaluated for entrapment efficiency, *In-vitro* release study, Kinetic data analysis,Stability study. The result from SEM analyses has confirmed the coating of surfactant on the surface of carrier. The formulation based maltodextrin showed higher entrapment efficiency of 82.64 ± 1.25 and *in-vitro* release of 98% at the end of 24hr was found to be best among the various formulations.The proniosome formulations were evaluated for FT-IR study, angle of repose and scanning electron microscopy and the result showed that the maltodextrin based formulation was best suited. Release was best explained by the zero order kinetics. Kinetic analysis shows that the drug release follows super case II transport diffusion.Maltodextrin based Proniosome formulation has showed appropriate stability for 90 days when compared with other carriers reconstituted niosomes by storing the formulation at refrigerator condition.

8. Madhav NVS et al., (2011) studied that Niosomes are formations of vesicles by hydrating mixture of cholesterol and nonionic surfactants. Different novel approaches used for delivering these drugs include liposomes, microspheres, nanotechnology, micro emulsions, antibody-loaded drug delivery, magnetic microcapsules, implantable pumps and niosomes. Niosomes and liposomes are equiactive in drug delivery potential and both increase drug efficacy as compared with that of free drug. Niosomes are preferred over liposomes because the former exhibit high chemical stability and economy. The application of vesicular (lipid vesicles and non-ionic surfactant vesicles) systems in cosmetics and for therapeutic purpose may offer several advantages. They improve the therapeutic performance of the drug molecules by delayed clearance from the circulation, protecting the drug from biological environment and restricting effects to target cells. This article focuses on the recent advances in niosomal drug delivery, potential advantages over other delivery systems, formulation methods, methods of characterization and the current research in the field of niosomes. Niosome appears to be a well preferred drug delivery system over liposome as niosome being stable and economic. Also niosomes have great drug delivery potential for targeted delivery of anti-cancer, antiinfective agents. Drug delivery potential of niosome can enhance by using novel concepts like proniosomes, discomes and aspasome. Niosomes also serve better aid in diagnostic imaging and as a vaccine adjuvant. Thus these areas need further exploration and research so as to bring out commercially available niosomal preparation.

9. Rajesh z. mujoriya et al., (2011) studied that niosomes represent a promising drug delivery module. They present a structure similar to liposome and hence they can represent alternative vesicular systems with respect to liposomes, due to the niosome ability to encapsulate different type of drugs within their multi environmental

structure. Niosomes are thoughts to be better candidate's drug delivery as compared to liposomes due to various factors like cost, stability etc. Various type of drug deliveries can be possible using niosomes like targeting, ophthalmic, topical, parentral, etc.

10. RekhaRao et al ., (2010) developed that a number of novel drug delivery systems have emerged encompassing various routes of administration to achieve controlled and targeted drug delivery. Encapsulation of the drug in vesicular structures is one such system, which can be predicted to prolong the existence of the drug in systemic circulation and reduce the toxicity, if selective uptake can be achieved. Consequently, a number of vesicular drug delivery systems such as liposomes, niosomes, transferosomes, ethosomes and proniosomes were developed. Proniosomes are water soluble carrier particles that are coated with surfactant and can be hydrated to form niosomal dispersion immediately before use on brief agitation in hot aqueous media. This review presents an overview about proniosomes reporting the preparation methods, characterization techniques and the studies of penetration and transport of various drugs through skin.

11. Astha Mishra et al., (2011) developed a drug delivery system which aims for delivering the therapeutic agent to the desired site of action.Pro-niosomes are promising drug carriers and are more advantageous than the conventional niosomes and liposomes. The advancements in the niosome lead to the evolution of proniosomal delivery systems. Proniosomes are non-ionicbased surfactant vesicles which may be hydrated immediately before use to yield aqueous niosome dispersions.They can incorporate both lipophilic as well as hydrophilic drugs. Proniosomal gel is basically used for the topical/transdermal applications.The givenarticle highlights all the salient features of a pro-niosomal gel, its advantages

over niosomes and liposomes, its method of preparation and its methods of characterization.

12. N.K. Jain et al.,(1998) developed a proniosome based transdermal drug delivery system of levonorgestrel (LN) was and extensively characterized both in vitro and in vivo. The proniosomal structure was liquid crystalline-compact niosomes hybrid which could be converted into niosomes upon hydration. The system was evaluated in vitro for drug loading, rate of hydration (spontaneity), vesicle size, polydispersity, entrapment efficiency and drug diffusion across rat skin. The effect of composition of formulation, amount of drug, type of Spans, alcohols and sonication time on transdermal permeation profile was observed. The stability studies were performed at 48C and at room temperature. The biological assay for progestational activity included endometrial assay and inhibition with the formation of corpora lutea. The study demonstrated the utility ofproniosomal transdermal patch bearing levonorgestrel for effective contraception. Ó 1998 Published by Elsevier Science.

13. Gannu P. Kumar et al., (2011) studied that vesicular systems are a novel means of drug delivery that can enhance bioavailability of encapsulated drug and provide therapeutic activity in a controlled manner for a prolonged period of time. Liposomes were the first such system but they suffer from a number of drawbacks including high cost and lack of stability at various pH. Niosomes are a nonionic surfactant vesicular system, which can be easily and reliably made in the laboratory. Many factors affect noisome formation such as the method of manufacture, nature of surfactant and encapsulated drug, temperature at which the lipids are hydrated and the critical packing parameter. This review describes all aspects of niosomes including their different compositions, the various methods of preparation, the effect of changing manufacturing parameters, methods of characterization, factors that affect

their stability, their use by various routes of administration, their therapeutic applications and the most important patents. The review also provides detailed information of the various types of niosomes that provide effective drug delivery.

14. **D. Akhilesh et al ., (2011)** studied that niosomes are non-ionic surfactant vesicles obtained on hydration of synthetic nonionic surfactants, with or without incorporation of cholesterol or other lipids. They are vesicular systems similar to liposomes that can be used as carriers of amphiphilic and lipophilic drugs. Niosomes are promising vehicle for drug delivery and being non-ionic, it is less toxic and improves the therapeutic index of drug by restricting its action to target cells. This systemic review article deals with preparation methods, characterizations, factors affecting release kinetic, advantages, and applications of niosomes. They are lamellar structures that are microscopic in size. They are now widely used as alternative to liposomes. Niosomal dispersion in an aqueous phase can be emulsified in anon-aqueous phase to regulate the delivery rate of drug and administer normal vesicle in external nonaqueous phase. Stable niosome dispersion must exhibit a constant particle size and a constant level of entrapped drug. Span 60 is the better surfactant of all because it is having high phase transition temperature and low HLB (Hydrophilic Lipophilic Balance) so it will form vesicles of good size.one more reason for the selection of span 60 and that was the critical packing factor which is between 0.5 and 1 for this surfactant so it forms spherical vesicles. If CPP factor is below 0.5 it cause micelles to form and if it was above 1 it will form inverted vesicles.

15. Madhav NVS et al (2011) developed thatniosomes are formations of vesicles by hydrating mixture of cholesterol and nonionic surfactants. Different novel approaches used for delivering these drugs include liposomes, microspheres, nanotechnology, micro emulsions, antibody-loaded drug delivery, magnetic

microcapsules, implantable pumps and niosomes. Niosomes and liposomes are equiactive in drug delivery potential and both increase drug efficacy as compared with that of free drug. Niosomes are preferred over liposomes because the former exhibit high chemical stability and economy. The application of vesicular (lipid vesicles and non-ionic surfactant vesicles) systems in cosmetics and for therapeutic purpose may offer several advantages. They improve the therapeutic performance of the drug molecules by delayed clearance from the circulation, protecting the drug from biological environment and restricting effects to target cells. This article focuses on the recent advances in niosomal drug delivery, potential advantages over other delivery systems, formulation methods, methods of characterization and the current research in the field of niosomes. Niosome appears to be a well preferred drug delivery system over liposome as niosome being stable and economic. Also niosomes have great drug delivery potential for targeted delivery of anti-cancer, antiinfective agents. Drug delivery potential of niosome can enhance by using novel concepts like proniosomes, discomes and aspasome. Niosomes also serve better aid in diagnostic imaging and as a vaccine adjuvant. Thus these areas need further exploration and research so as to bring out commercially available niosomal preparation.

16. Alhatbabita et al., (2013) reviewed that an ideal drug delivery system delivers drug at rate dictated by the need of the body over the period of treatment and it channels the active entity solely to the site of action. Drug targeting can be defined as the ability to direct a therapeutic agent specifically to desired site of action with little or no interaction with nontarget tissue. Different novel approaches used for delivering these drugs include liposomes, microspheres, nanotechnology, micro emulsions, antibody loaded drug delivery, magnetic microcapsules, implantable pumps and niosomes. Niosomes are vesicles composed of non-ionic surfactants, which are

biodegradable, relatively nontoxic, more stable and inexpensive, an alternative to liposomes. They present a structure similar to liposome and hence they can represent alternative vesicular systems with respect to liposomes, due to the niosome ability to encapsulate different type of drugs within their multi environmental structure. Niosomes are thoughts to be better candidate's drug delivery as compared to liposomes due to various factors like cost, stability etc.

17. ShwetaPatidar et al.,(2012) formulated and evaluated Niosomes, a synthetic microscopic vesicles consisting of an aqueous concentration is enclosed in a bilayer consisting of cholesterol and nonionic surfactants to improve the low corneal permeability for effective management trigeminal neuralgia. Proniosomal gels of flupirtine Maleate were developed with span 20, span 60, span 80, tween 20 and tween 80 with cholesterol. Nonionic surfactant vesicles formed immediately upon hydrating proniosomal gel. The morphological characteristics, entrapment efficiency, In-vitro drug release, drug release kinetic, ocular irritation test, In vivo studies for Trigeminal neuralgia was determined. The entrapment efficiency (EE %) of flupirtine maleate was determined by centrifugation of freeze thawed vesicles followed the order span80 >span 60> span 20> tween 20> tween 80. The In vitro drug release studies showed that there was a prolong release of drug which followed Higuchi model. Niosome formed from span 80 and cholesterol is promising approach to prolong antinociception activity and improve permeation rate as compared to pure drug.

18. Singh Neelam et al., (2010) formulated ceftriaxone sodium which is a third generation cephalosporin. This antibiotic cannot be absorbed orally owing to its very less permeation through GI epithelia. Other problem associated with the drug is its acid labile nature. The present study attempts to increase the intestinal permeability of

BCS Class III drug ceftriaxone sodium by using certain intestinal absorption enhancers. The blend of permeation enhancer, drug, and other excipients were incorporated into Beads to formulate the final dosage form. To enhance the permeation, intestinal permeation enhancers were used in various molar ratios with the drug. The effect of absorption enhancers on the lipophilicity of ceftriaxone sodium was determined by means of the n-octanol/water system. The changes in partition coefficient by the octanol/water system were confirmed using an in vitro transport model with excised animal intestinal membrane. The results indicated that there is significant improvement in the permeability of the drug and the extent of enhancement was highly dependent on the type of used absorption enhancer. Permeation enhancer and drug were formulated into beads further evaluated for permeability by using biological membrane. The release profile of ceftriaxone from beads was observed in both gastric and intestinal pH (7.4 buffer). Release of drug from the beads in both the media was found to occur predominantly by diffusion following non Fickian transport mechanism and was higher and more rapid in intestinal pH than in gastric pH. The results obtained from this study indicate that ceftriaxone sodium could be successfully delivered orally when formulated with permeation enhancers.

19. YogeshGarg et al., (2010) developed a mucoadhesive multiparticulate sustained drug delivery system of pravastatin sodium, a highly water-soluble and poorly bioavailable drug, unstable at gastric pH Mucoadhesive microparticles were formulated using eudragit S100 and ethyl cellulose as mucoadhesive polymers. End-step modification of w/o/o double emulsion solvent diffusion method was attempted to improve the purity of the product, that can affect the dose calculations of sustained release formulations and hence bioavailability. Microparticles formed were discrete,

free flowing, and exhibited good mucoadhesive properties. DSC and DRS showed stable character of drug in microparticles and absence of drug polymer interaction. The drug to polymer ratio and surfactant concentration had significant effect on mean particle size, drug release, and entrapment efficiency. Microparticles made with drug: eudragit S100 ratio of 1:3 (F6) exhibited maximum entrapment efficiency of 72.7% and ex vivo mucoadhesion time of 4.15 h. In vitro permeation studies on goat intestinal mucosa demonstrated a flux rate (1,243 μg/cm2/h) that was 169 times higher than the flux of pure drug. The gastric instability problem was overcome by formulating the optimized microparticles as enteric-coated capsules that provided a sustained delivery of the highly water-soluble drug for 12 h beyond the gastric region. The release mechanism was identified as fickian diffusion (n=0.4137) for the optimized formulation F6. Conclusively, a drug delivery system was successfully developed that showed delayed and sustained release up to 12 h and could be potentially useful to overcome poor bioavailability problems associated with pravastatin sodium.

AIM AND PLAN OF WORK

Aim:

The aim of present study is to design and formulate proniosome based niosomal suspension of *Bosentan monohydrate* with increased bioavailability and sustained release.

The important objectives of the proposed research work are:-

- To carry out pre-formulation study of excipients and their compatibility with the API.
- To increase the bioavailability of *Bosentan monohydrate*
- Determination of λ_{max} for*Bosentan monohydrate*
- To carry out preliminary investigation studies with different process variables to arrive at an optimum formulation revealing particle size suitable for oral delivery
- Selection and optimization of the best formulation
- To prepare niosomal formulation of *Bosentan monohydrate* with cholesterol and span
- To perform stability studies on the most satisfactory formulation.
- To reduce the dose related side effects which are caused by *Bosentan monohydrate* long term therapy
- To reduce total dose amount of the drug in the formulation

PLAN OF WORK

I. Preformulation studies

1. Solubility analysis

2. IR spectroscopy

3. Melting point determination

II. Compatibility studies by IR-Spectroscopy

III. Standard calibration curve of Bosentan monohydrate

IV. Percentage entrapment efficiency

V. Vesicle size and morphology

VI. Characterization of proniosomal suspension.

A. Ex-vivo drug permeation study

B. Release kinetics

MATERIAL AND METHODS

4.1List of equipment

Sl.no	Equipment	Manufacturer
1	Mechanical stirrer	Remimotors
2	magnetic stirrer	Remi motors
3	Hot air oven	universal
4	pH meter	GeNej
5	Deep freezer	Science tech
6	PC based double beam spectrophotometer-2202	Systronic
7	microscope	Olympus India PVT LTD (Ch20iBRMP) Noida
8	Centrifuse	Remi
9	Water bath	Science tech
10	Vortex	Remi CM101
11	Digital balance	Axis

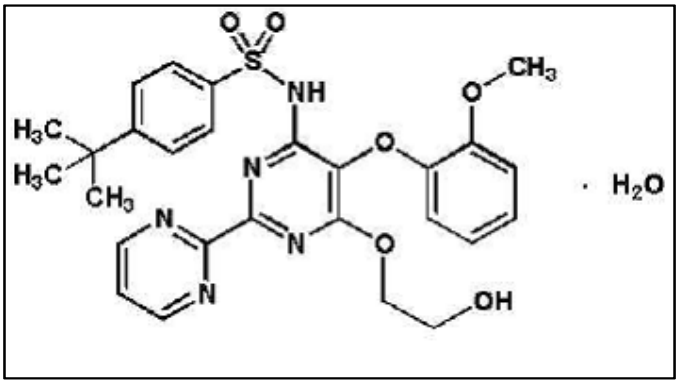
4.2List of chemicals

Sl. No.	Chemical name	Manufacturer
1	Bosentan monohydrate	Parabolic Drugs Ltd., Chandigarh
2	Methanol	Worli Mumbai
3	Ethanol	Jiangsu huaxiinternationaltrade Co Ltd. CHINA
4	Cholesterol	Himedia, Mumbai
5	Span 20	SD Fine, Mumbai
6	Acetone	Rankem, New Delhi
7	N hexane	Merke, Mumbai
8	Pet ether	Merke Mumbai
9	Disodium hydrogen phosphate	Merke Mumbai
10	Dimethyl sulphoxide(DMSO)	Merke Mumbai
11	potassium dihydrogen phosphate	Merke Mumbai
12	Sodium chloride	Merke Mumbai
13	Chloroform	Rankem, New Delhi

4.3 DRUG PROFILE

Drug description

Tracleer is the proprietary name for bosentan, an endothelin receptor antagonist that belongs to a class of highly substituted pyrimidine derivatives, with no chiral centers. It is designated chemically as 4-tert-butyl-N-[6-(2-hydroxy-ethoxy)-5-(2-methoxy-phenoxy)[2,2´]-bipyrimidin-4-yl]- benzenesulfonamide monohydrate and has the following structural formula:



Molecular weight- 569.64

Molecular formula- $C_{27}H_{29}N_5O_6S•H_2O$

Description-Bosentan is a white to yellowish powder.

Solubility- It is poorly soluble in water (1.0 mg/100 mL) and in aqueous solutions at low pH (0.1 mg/100 mL at pH 1.1 and 4.0; 0.2 mg/100 mL at pH 5.0). Solubility increases at higher pH values (43 mg/100 mL at pH 7.5). In the solid state, bosentan is very stable, is not hygroscopic and is not light sensitive.

Dose- In adult patients 62.5 mg twice daily for 4 weeks and then increased to the maintenance dose of 125 mg twice daily for oral administration.

For pediatric patients aged 2 years or older- 2 mg/kg body weight twice daily(for oral administration)

Excipients: corn starch, pre-gelatinized starch, sodium starch glycolate, povidone, glycerylbehenate, magnesium stearate, hydroxypropylmethylcellulose, triacetin, talc, titanium dioxide, iron oxide yellow, iron oxide red, and ethylcellulose.

Melting point-195-198°C

Standards- 99.0% to 102.0%

Storage- Preserved in a well closed container. Keep in cool and dry place.

Pharmacokinetic properties

The pharmacokinetics of Bosentan has mainly been documented in healthy subjects. Limited data in patients show that the exposure to Bosentan in adult pulmonary arterial hypertension patients is approximately 2-fold greater than in healthy adult subjects.

Bosentan displays dose and time-dependent pharmacokinetics. Clearance and volume of distribution decrease with increased intravenous doses and increase with time. After oral administration, the systemic exposure is proportional to dose up to 500 mg. At higher oral doses, C_{max} and AUC increase less than proportionally to the dose.

Absorption

In healthy subjects, the absolute bioavailability of bosentan is approximately 50% and is not affected by food. The maximum plasma concentrations are attained within 3–5 hours.

Following a single 125 mg oral dose of bosentan in healthy volunteers, the Tmax, Cmax and half-life of bosentan were 4 h, 1500 ng/ml and 6 h, respectively.

Reference: Tracleer (Bosentan) 125 mg FDA's NDA submission: 49-073, Clinical Pharmacology and Biopharmaceutics Review.

Distribution

Bosentan is highly bound (> 98%) to plasma proteins, mainly albumin. Bosentan does not penetrate into erythrocytes. A volume of distribution of about 18 litres was determined after an intravenous dose of 250 mg.

Biotransformation and elimination

After a single intravenous dose of 250 mg, the clearance was 8.2 L/h. The terminal elimination half-life ($t_{1/2}$) is 5.4 hours. Upon multiple dosing, plasma concentrations of Bosentan decrease gradually to 50%–65% of those seen after single dose administration. This decrease is probably due to auto-induction of metabolizing liver enzymes. Steady-state conditions are reached within 3–5 days. Bosentan is eliminated by biliary excretion following metabolism in the liver by the cytochrome P450 isoenzymes, CYP2C9 and CYP3A4. Less than 3% of an administered oral dose is recovered in urine. Bosentan forms three metabolites and only one of these is pharmacologically active. This metabolite is mainly excreted unchanged via the bile. In adult patients, the exposure to the active metabolite is greater than in healthy subjects. In patients with evidence of the presence of cholestasis, the exposure to the active metabolite may be increased. Bosentan is an inducer of CYP2C9 and CYP3A4 and possibly also of CYP2C19 and the P-glycoprotein.

In vitro, Bosentan inhibits the bile salt export pump in hepatocyte cultures.In vitro data demonstrated that Bosentan had no relevant inhibitory effect on the CYP isoenzymes tested (CYP1A2, 2A6, 2B6, 2C8, 2C9, 2D6, 2E1, 3A4). Consequently, Bosentan is not expected to increase the plasma concentrations of medicinal products metabolized by these isoenzymes

Bosentan is a dual endothelin receptor antagonist (ERA) with affinity for both endothelin A and B (ETA and ETB) receptors. Bosentan decreases both pulmonary and systemic vascular resistance resulting in increased cardiac output without increasing heart rate. It is designated chemically as 4-tert-butyl-N-[6-(2-hydroxy-ethoxy)-5-(2-methoxy-phenoxy)[2,2´]-bipyrimidin-4-yl]-benzenesulfonamide monohydrate and has the following structural formula.

Mechanism of action

Bosentan is a dual endothelin receptor antagonist (ERA) with affinity for both endothelin A and B (ETA and ETB) receptors. Bosentan decreases both pulmonary and systemic vascular resistance resulting in increased cardiac output without increasing heart rate.

The neurohormone endothelin-1 (ET-1) is one of the most potent vasoconstrictors known and can also promote fibrosis, cell proliferation, cardiac hypertrophy and remodelling, and is pro-inflammatory. These effects are mediated by endothelin binding to ETA and ETB receptors located in the endothelium and vascular smooth muscle cells. ET-1 concentrations in tissues and plasma are increased in several cardiovascular disorders and connective tissue diseases, including pulmonary arterial hypertension, scleroderma, acute and chronic heart failure, myocardial ischaemia, systemic hypertension and atherosclerosis, suggesting

apathogenic role of ET-1 in these diseases. In pulmonary arterial hypertension and heart failure, in the absence of endothelin receptor antagonism, elevated ET-1 concentrations are strongly correlated with the severity and prognosis of these diseases.

Bosentan competes with the binding of ET-1 and other ET peptides to both ETA and ETB receptors, with a slightly higher affinity for ETA receptors (Ki = 4.1–43 nanomolar) than for ETB receptors (Ki = 38-730 nanomolar). Bosentan specifically antagonises ET receptors and does not bind to other receptors.

Side effects- There are some side effects of Bosentan monohydrate which must not be neglected while using this drug.

- Nausea
- stomach pain
- loss of appetite
- Dark urine, clay-coloured stools or
- Jaundice (yellowing of the skin or eyes).

Less serious side effects may include

- Headache and dizziness
- flushing
- swelling of the feet, ankles, or legs
- upset stomach
- fatigue

Precautions when taking Bosentan

Before taking Bosentan, tell your doctor or pharmacist if you are allergic to it, or if you have any other allergies. This product may contain inactive ingredients, which can cause allergic reactions or other problems. Talk to your pharmacist for more details.

Before using this medication, tell your doctor or pharmacist your medical history, especially of liver disease, anaemia.This drug may make you dizzy. Do not drive, use machinery, or do any activity that requires alertness until you are sure you can perform such activities safely. Avoid alcoholic beverages since they can increase the effects of dizziness and also increase the risk of serious liver problems.

This medication must not be used during pregnancy. It may harm an unborn baby. If you become pregnant

Indications

Pulmonary Arterial Hypertension

Tracleer is indicated for the treatment of pulmonary arterial hypertension (PAH) (WHO Group 1) to improve exercise ability and to decrease clinical worsening. Studies establishing effectiveness included predominantly patients with NYHA Functional Class II-IV symptoms and etiologies of idiopathic or heritable PAH (60%), PAH associated withconnective tissue diseases (21%), and PAH associated with congenital heart disease with left-to-right shunts (18%).

5.4 PROFILES FOR EXCIPIENTS

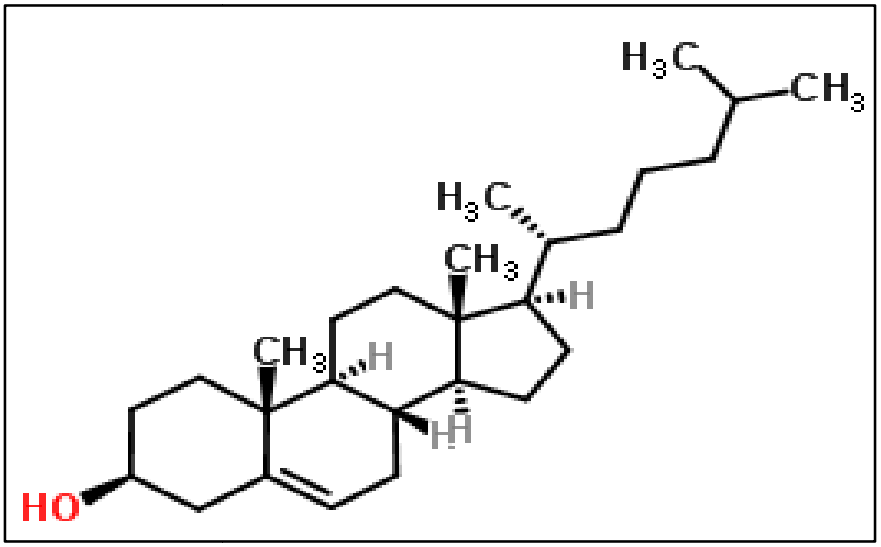
Cholesterol

Synonym: Cholesterin, Cholesterolum.

Molecular formula: $C_{22}H_{46}O$

Molecular weight: 386.67

Structural formula:



Description: Cholesterol occurs as white or faintly yellow, almost odorless powder or granules which turns yellow to tan colouron prolonged exposure to light and air.

Assay: 95.0-97.0%

Melting point: 150ºC.

Solubility: Soluble in acetone, chloroform, ethanol, methanol, hexane, and ether and practically insoluble in water.

Storage:	Stored in a well-closed container, proctected from light.
Functional category:	Emollient and Emulsifying agent
Applications:	As an emulsifying agent, it imparts water-absorbing power to an ointment and also used as an emollient in varying concentrations.
Safety:	Cholesterol is essentially non-toxic and non-irritant material at the level employed asan excipient.

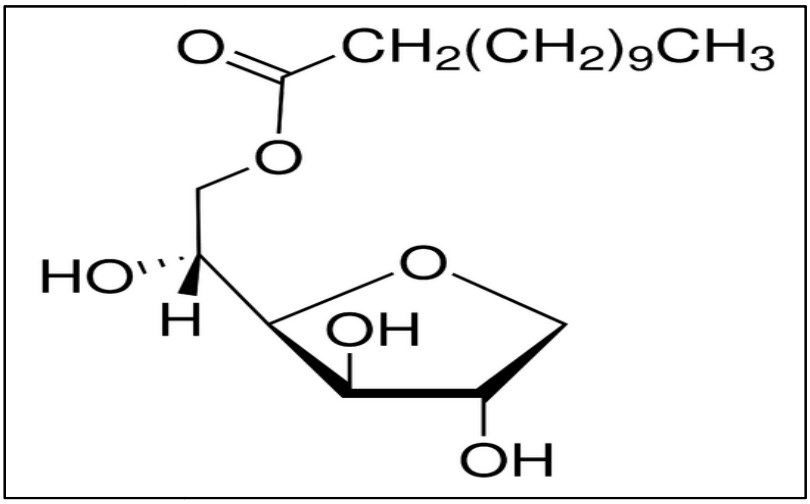
Sorbitanmonosterate

Synonym: Span 20

Category: Non-ionic surface active agent.

Molecular formula: $C_{18}H_{34}O_6$

Structural formula:



Molecular weight: 346.46

Density: 1.032 g/mL at 25 °C(lit.)

Description: amber to sepia oil liquids, non-toxic, odorless.

Solution, a small amount of dissolved in isopropyl alcohol and xylene.

Functional category: Emulsifying agent, non-ionic surfsctsnt, solubilizing agent, wetting agent and dispersing agent

Solubility: Soluble in most organic solvents. In water although insoluble, they are dispersible

Storage:	it should be stored in a well closed container in a cool and dry place
Safety:	widely used in cosmetics, food products and topical pharmaceutical formulations and are regarded as nontoxic and nonirritant material
Application:	Used in pharmaceutical formulations as emulsifying agent in the preparation of creams, emulsion and ointment for topical application.

4.5. Materials and methods

A. Materials

Bosentan Monohydrate was a gift pack from "Parabolic Drugs LTD." which is an ISO14001 – 2004 certified company(INDIA). Sorbitanmonosterate (span 20), and cholesterol (Chol; >99%) were purchased fromHimedia, Mumbai (INDIA).All other chemicals and solventswere of good quality, purchased from different certified Indian companies which is been mentioned earlier.

B. Preparation of pro-niosomes

Pro-niosomes were prepared by the method reported by Voraet al. (1998) with some modifications.

- In glass vials accuratelyweighed amounts of the surface-active agent were mixed with the appropriate amount of cholesterol to make 1 mmol total lipids.The amounts of cholesterol were added as 10% incrementsvaried from 0% to 60% of total lipids.
- Bosentan monohydrate was added to thenonionic surfactant/cholesterol mixture.
- Absolute ethanol (about400mg) was added to the mixtures then vials were tightly sealedand warmed in water bath (55–60~C) for5min while shaking untilcomplete dissolution of cholesterol.
- To each of the formedparent solutions, about 0.16 ml hot distilled water (55–60~C) wasadded while warming in the water bath for 3–5 min till a clear ortranslucent solution was produced.

- The mixtures were allowed tocool down at room temperature and observed for the formation oftransparent solution, two phases liquid, and translucent, transparent or white creamy pro-niosomal gel.To obtaincharged pro-niosomes, SA or DCP were added to lipid mixtures thendissolved into ethanol as described above. SA or DSP did not affectthe final appearance of the developed proniosomes.
- The obtainedformulations were kept in the same closed glass vials in dark for further characterization.

C. Hydration step and formation of niosomes

Niosomes were prepared by hydration of the gels prepared asdescribed above. About 7 ml of phosphate buffer (pH7.4) was added into each vial followed by heating for 10 min at atemperature above 60~C in a water bath. Vortexing of the formulations was done two to three times during the 10 min heating. The final volume was adjusted to 10 ml by the same buffer.

Formulations	Cholesterol (mmol)	Nonionic surfactant (mmol)	Bosenan monohydrate(mg)	Ethanol (mg)	Water
F3	0.2	0.8	20	400	0.16
F4	0.3	0.7	20	400	0.16
F5	0.4	0.6	20	400	0.16
F6	0.5	0.5	20	400	0.16
F7	0.6	0.4	20	400	0.16
F8	0.7	0.3	20	400	0.16
F9	0.8	0.2	20	400	0.16

Characterization of niosomal preparations

A. Microscopic examination

Small amounts of the formed niosomes were spread on a glassslide and examined for the vesicles structure and the presence ofinsoluble drug crystals using ordinary light microscope with varied magnification powers (10× and 40×). Also the size of niosomes was analyzed under Trinocular microscope usingocular micrometer and varnier caliper.Photomicrographs werealso taken for niosomes by TEM analysis to conform the structure and size of niosomes.

B. Solubility determination of Bosentan monohydrate

An excess amount of Bosentan monohydrate was added to each of distilledwater, and phosphate buffer (pH 5.5, 6.5, 6.8, 7.4, and 8). The mixtures were then kept at ambient temperature for 72 h in a shaker water bath to get equilibrium. The equilibrated samples were centrifuged at 3000 rpm for5min. Aliquot portions of the supernatants were taken and properly diluted with phosphate buffer (pH 7.4) for quantification ofBosentan monohydratespectrophotometrically at 270 nm.

C. Determination of entrapment efficiency ofBosentan monohydrate in niosomes

Dialysis method*(Udupa et al., 1993).*

From the niosomal dispersion formed from pro-niosomes of Span 20,unentrapped free drug was removed by placing 1 ml of the dispersion into a glass tube to which a cellophane membrane was attached to one side

and dialyzing exhaustively for 1 h each timeagainst 100 ml of phosphate buffer (pH 7.4). The dialysis of free Bosentan monohydrate was completed after about six changes of buffer solutionwhere no further Bosentan monohydrate could be detected in the solution. Thedrug content was determined spectrophotometrically at 270 nmagainst phosphate buffer (pH 7.4) as a blank. Amount of entrappeddrug was obtained by subtracting amount of unentrapped drug from the total drug incorporated (Deepika and Indu, 2005).

$$\textbf{Entrapment efficiency} = \frac{\textbf{amount of drug entrapped}}{\textbf{total amount of drug added}} \times \textbf{100}$$

Freeze thawing/centrifugation method

1ml sample of prepared niosome was frozen for 24h at -20^{O}C in Eppendorf tube. The frozen sample were removed fromthe freezer and let to thaw at room temperature, then centrifuged at 14,000 rpm for 40 min at 4^{O}C. Niosomal pellets were resuspended in phosphate buffer (pH 7.4) and then centrifuged again.This washing procedure was repeated two times to ensure thatthe unentrapped drug was no longer present in the void volumebetween the niosomes. The supernatant was separated each timefrom niosomal pellets and prepared for the assay of free drug.Each result was the mean of three determinations(±S.D.). The % entrapped Bosentan monohydrate was calculated as follows

$$\textbf{Entrapment efficiency} = \frac{\textbf{amount of drug entrapped}}{\textbf{total amount of drug added}} \times \textbf{100}$$

Assessment of Bosentan monohydrate release rates from niosomes

The niosomal pellets prepared from proniosomes of Span 20were separated by centrifugation and washed twice as describedabove. The pellets were resuspended in 50 ml of phosphate buffer(pH 7.4). The suspension was placed in a 125 ml stoppered glassflask in a shaking water bath at 37~C. 1 ml samples were with-drawn at the following intervals: 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12hrafter incubation. Samples were centrifuged as mentioned beforeand supernatants were assayed at 270 nm. The percentage of drugrelease was plotted as a function of time(Sammour and Hassan, 1996).

D. Ex-vivo permeation studies

The rate of permeation of drugs from pro-niosomal formulations can be determined by using Franz diffusion cell.The drug release study of niosomes performed by Franz diffusion cell" consisted of a hollow glass cylindermade up of borosil glass. One end of the cylinder was covered with goat intestine. The diffusion cell consists of two compartments (donar and receptor) which were placed in a 250 ml borosil beaker.

The content of diffusion cell was agitated with the help of a glass stirrer. Thereceptor cell contained a magnetic bead and was rotated at a constant speed. The temperature in a donar and receptor cells was maintained at 37±0.5ºC with the help of thermostat. Two milliliters of each formulation was subjected to release studies. Phosphate buffer (20 ml) pH 7.4 was placed in the receptor cell. Two milliliters sample of each formulation was transferred to the dissolution cell.

One milliliter sample was withdrawn from the receptor cell at specified time intervals. At each time immediately after the removal of the sample, the medium was compensated with fresh phosphate buffer (pH 7.4). The samples were analyzed for Bosentan Monohydrate content using a UV spectrophotometer (PC based double beam Systronic UV spectrophotometer 2202) at λ max 270 nm.

E. Statistical analysis

The data were reported as mean± S.D.*(n* = 3) and statistical analysis of the data was carried out using one way ANOVA followed by LSD test at a level of significant of $P < 0.05$.

RESULTS AND DISCUSSION

5.1. Preformulation studies:

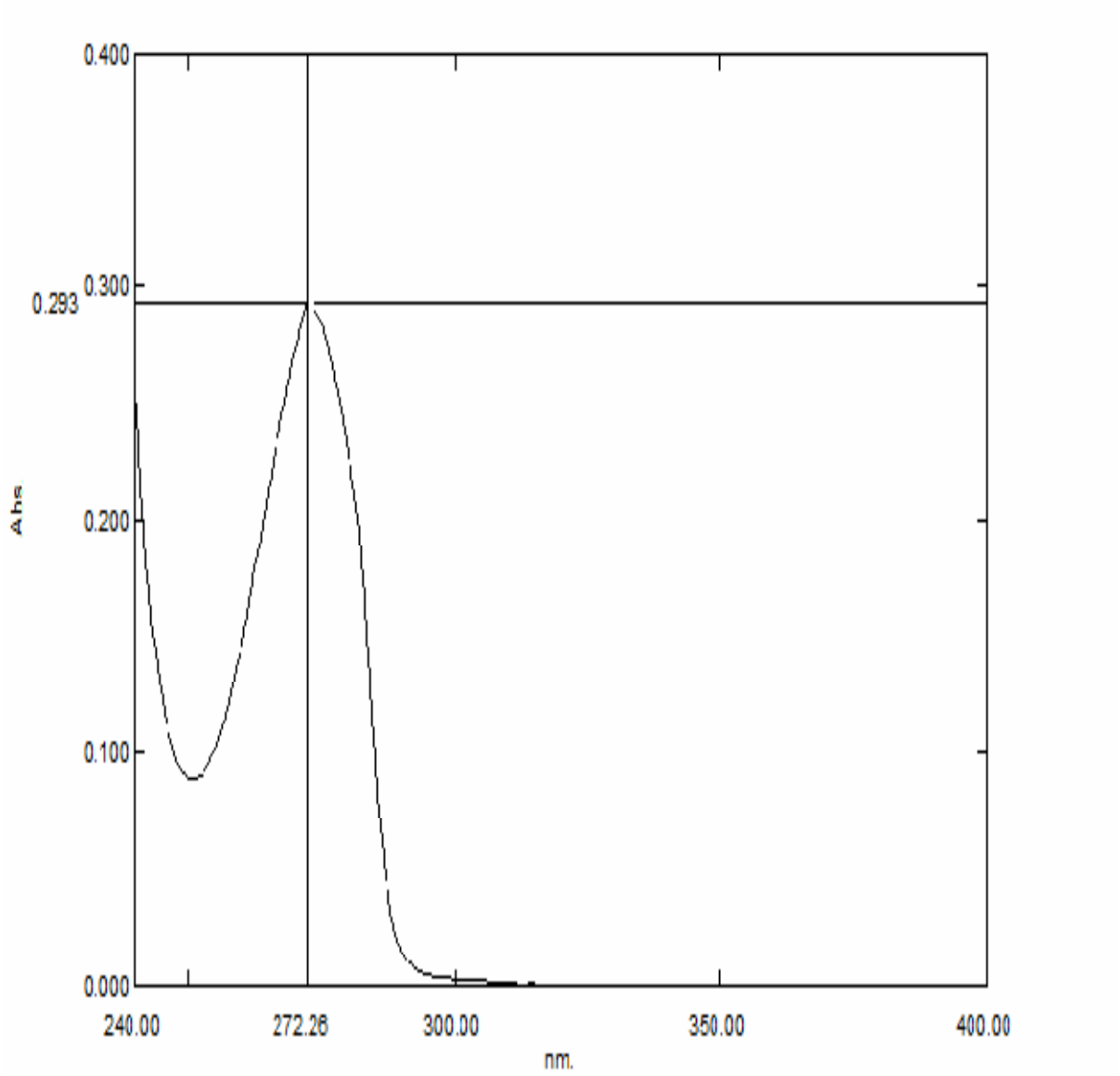
5.1.1. Solubility analysis:

Table 5.1: Solubility of *Bosentan monohydrate* in various solvent

Name of solvents	Solubility
Water	Practically insoluble
Pbs (6.8)	Slightly soluble
Pbs (7.4)	Practically insoluble
DMSO	Soluble
Ethanol	Soluble
Methanol	Slightly soluble
n-hexane	Practically insoluble
Acetone	Soluble
Pet ether	Practly insoluble
Chloroform	Soluble
Acetonitrile	Soluble
dichloromethane	Soluble

5.1.2a. determination of λ_{max}:

Fig.no:5.1: $\lambda_{max}$of Bosentan monohydrate



5.1.2b. IR spectroscopy:

Fig.no:5.2 FT-IR Spectra of Bosentan monohydrate

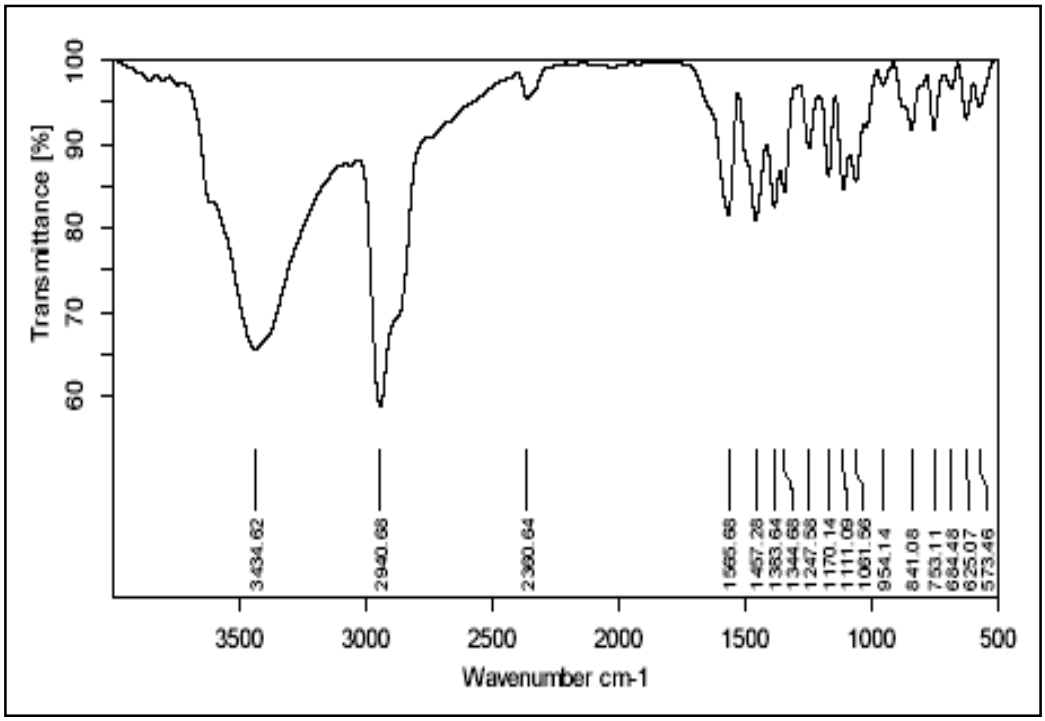


Fig.no:5.3 FT-IR Spectra of Bosentan monohydrate+ Cholesterol

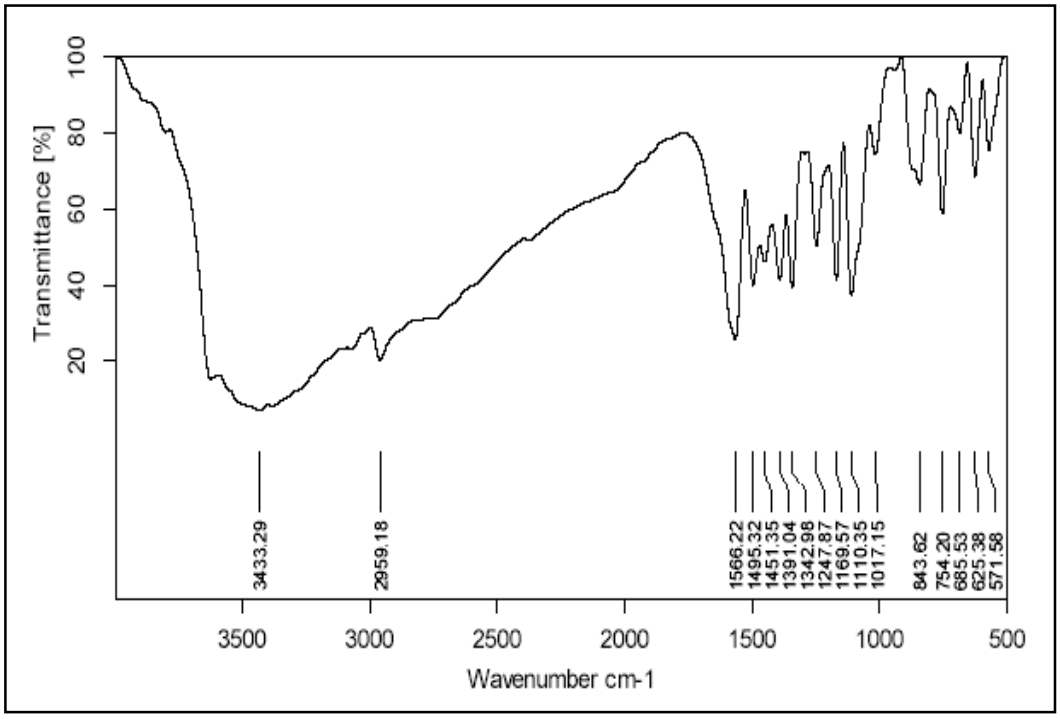


Table 5.2: FTIR Spectral analysis of pure drug

Sl no	Functional groups	Observed frequencies
1.	OH Stretching	3433-3000
2.	C-H stretching of CH_2	2959
3.	C=C stretching	1566
4.	O-H deformations vibrations	1495
5.	CH_2 in plane deformations	1451
6.	CH_3 deformation	1391
7.	CH_3 deformation	1342
8.	C-O stretching of carbonyl	1247
9.	C-O-C stretching	1169
10.	CH_2 out of bending vibration	1017

Table 5.3: FTIR Spectral analysis of Drug + cholesterol

Sl no	Functional groups	Observed frequencies
1.	OH Stretching	3434
2.	C-H stretching of CH_2	2940
3.	C=C stretching	1565
4.	O-H deformations vibrations	1457
5.	CH_3 deformation	1383
6.	CH_3 deformation	1344
7.	C-O stretching of carbonyl	1247
8.	C-O-C stretching	1170
9.	CH_2 out of bending vibration	1061

5.1.3. Melting point determination

The melting point of Bosentan monohydrate was found to be 196^{o}C which was found to be within the reported range of 195-198^{o}C. It complies with the standards thus indicate that, the sample was pure.

5.3. Standard calibration curve of *Bosentan monohydrate*

Table no:5.4Standard calibration curve of *Bosentan monohydrate*

S.no	Concentration (µg/ml)	Absorbance(nm) (In ethanol)	Absorbance(nm) (in pH 7.4 PBS+sls)
1	0	0.000	0.000
2	5	0.303	0.039
3	10	0.388	0.112
4	15	0.509	0.200
5	20	0.602	0.258
6	25	0.698	0.325
7	30	0.744	0.395
8	35	0.841	0.449
9	40	0.977	0.512
10	45	1.041	0.582
11	50	1.170	0.619

Fig.no:5.4Standard calibration curve of Bosentan monohydrate in ethanol

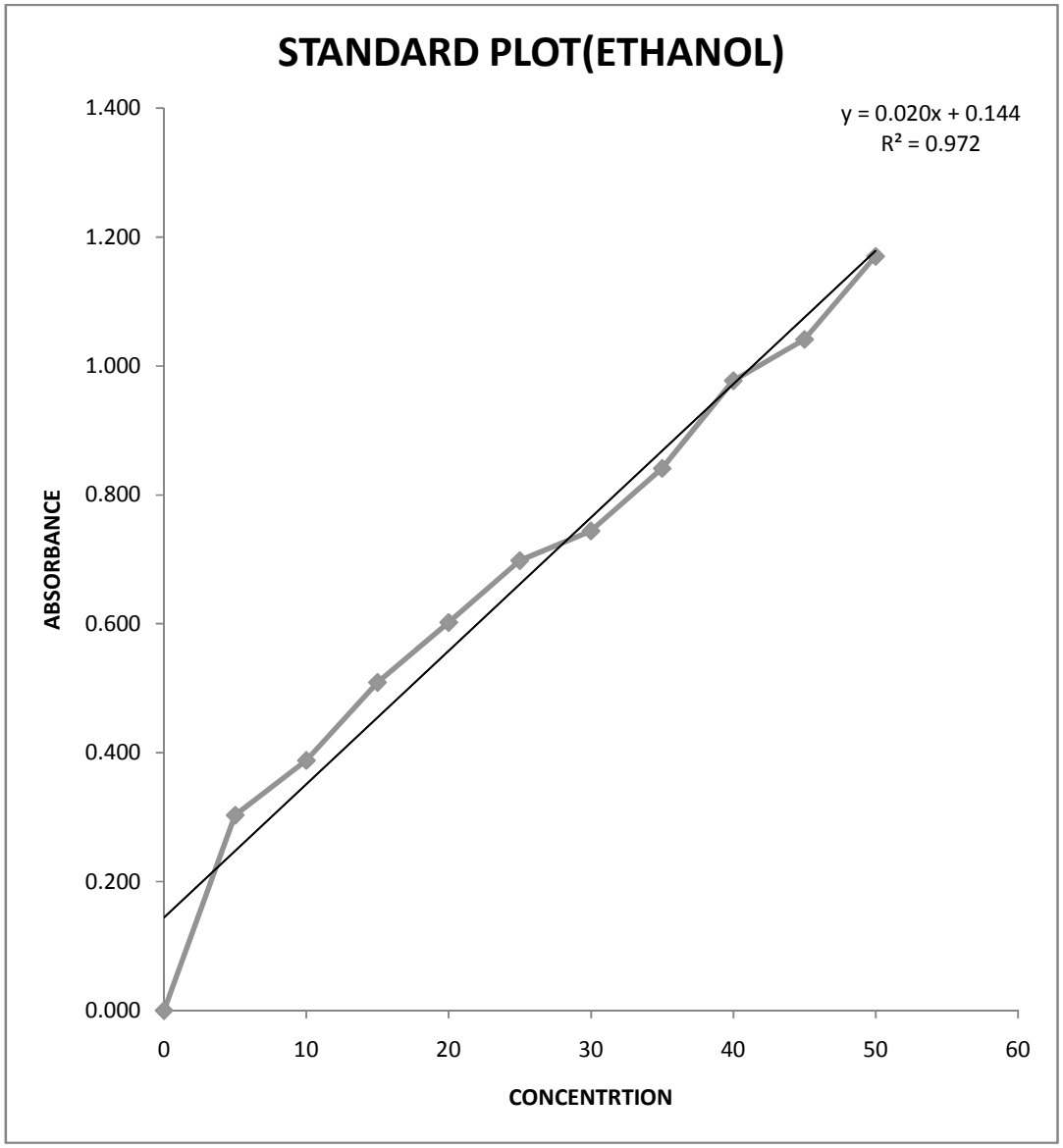
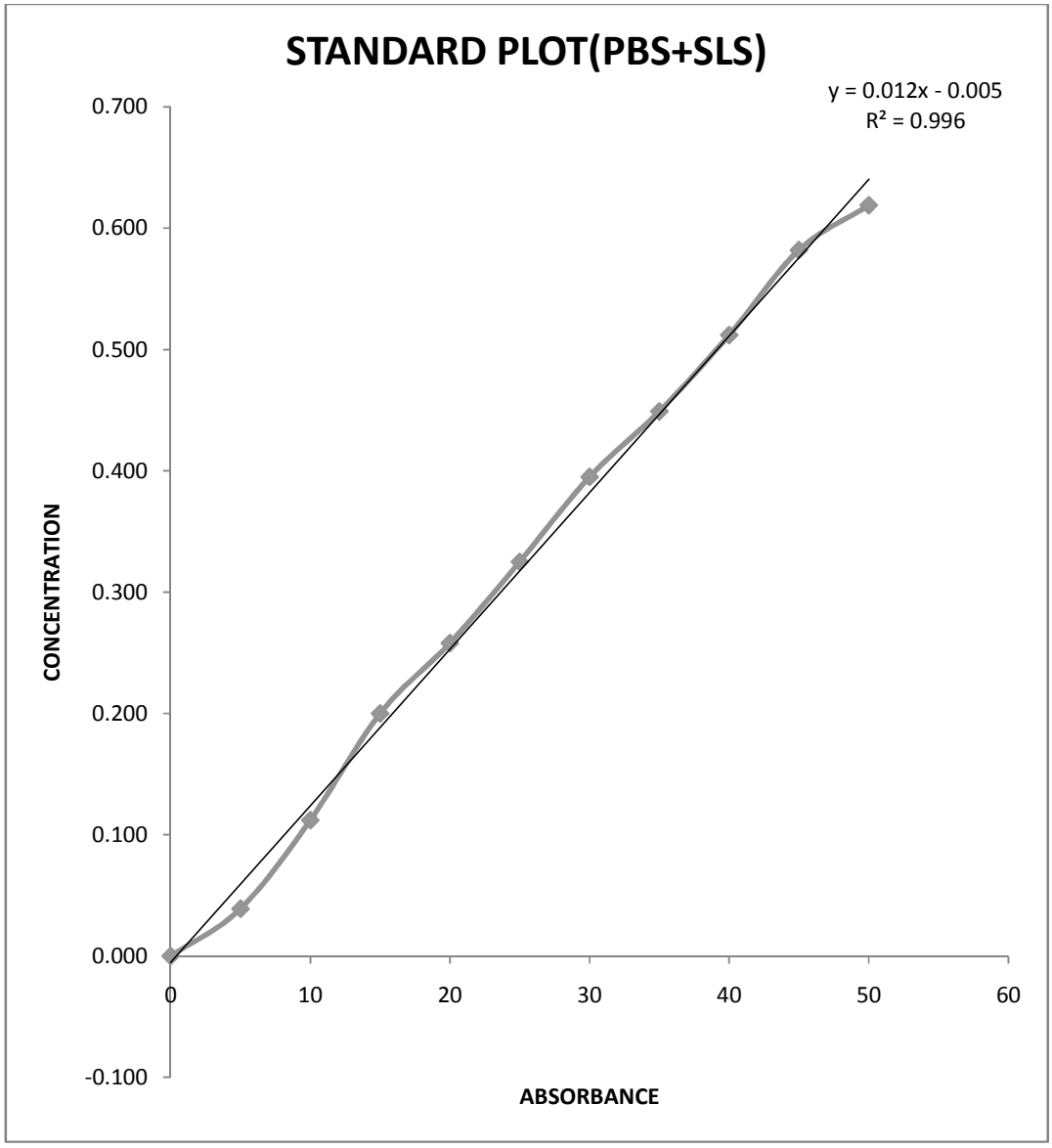


Fig.no:5.5 Standard calibration curve of Bosentan monohydrate in phosphate buffer +sodium lauryl sulphate

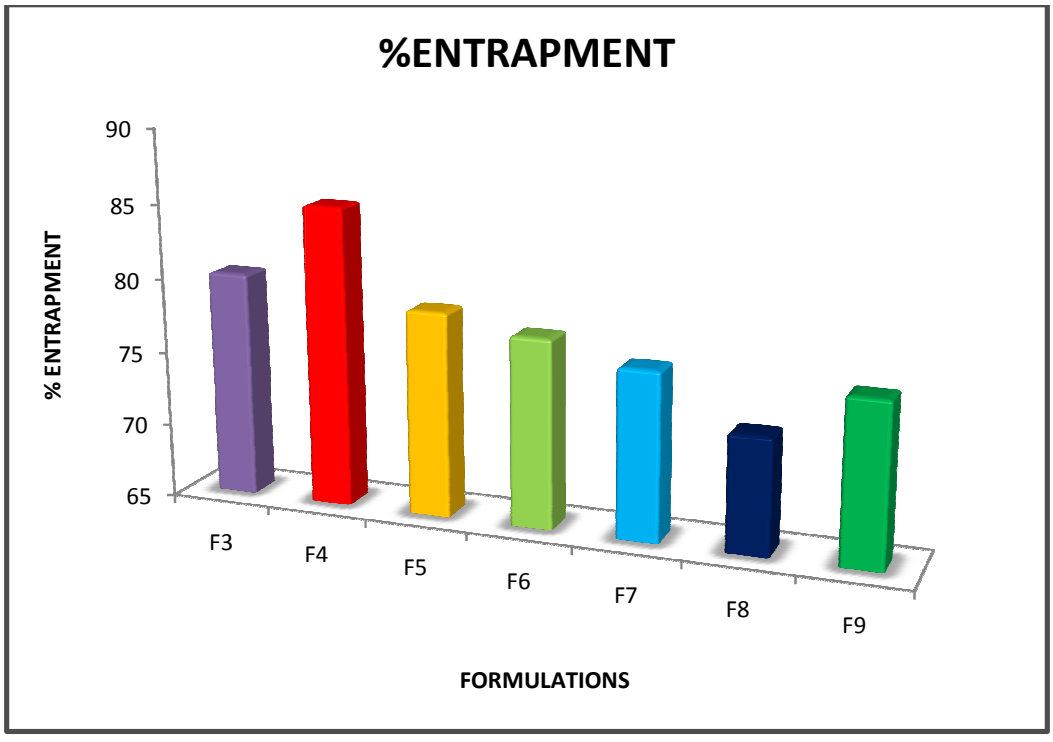


5.4 Percentage entrapment efficiency

Table no:5.5 Percentage entrapment efficiency of Bosentan monohydrate

FORMULATIONS	%ENTRAPMENT EFFICIENCY
F3	80.225
F4	85.198
F5	78.796
F6	77.717
F7	76.331
F8	72.817
F9	75.938

Fig no:5.6 Percentage drug entrapment efficiency of Bosentan monohydrate



5. Vesicle size and morphology

Fig no:5.7Microphoto graph of groups of noisome at 10X Magnification

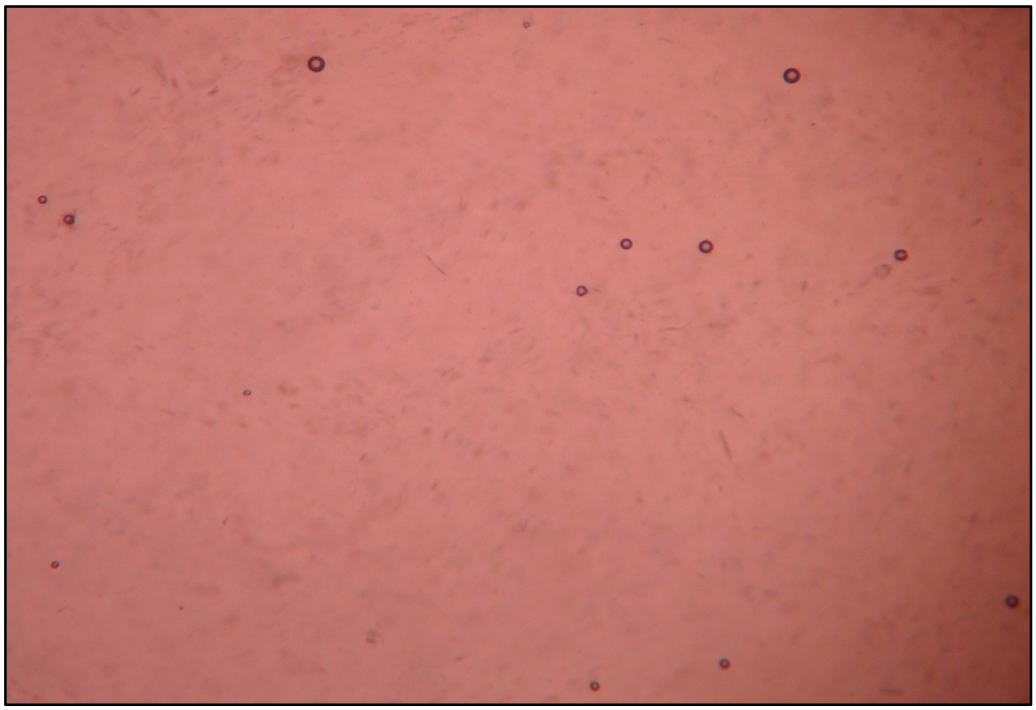


Fig no:5.8Microphoto graph of groups of noisome at 10X Magnification

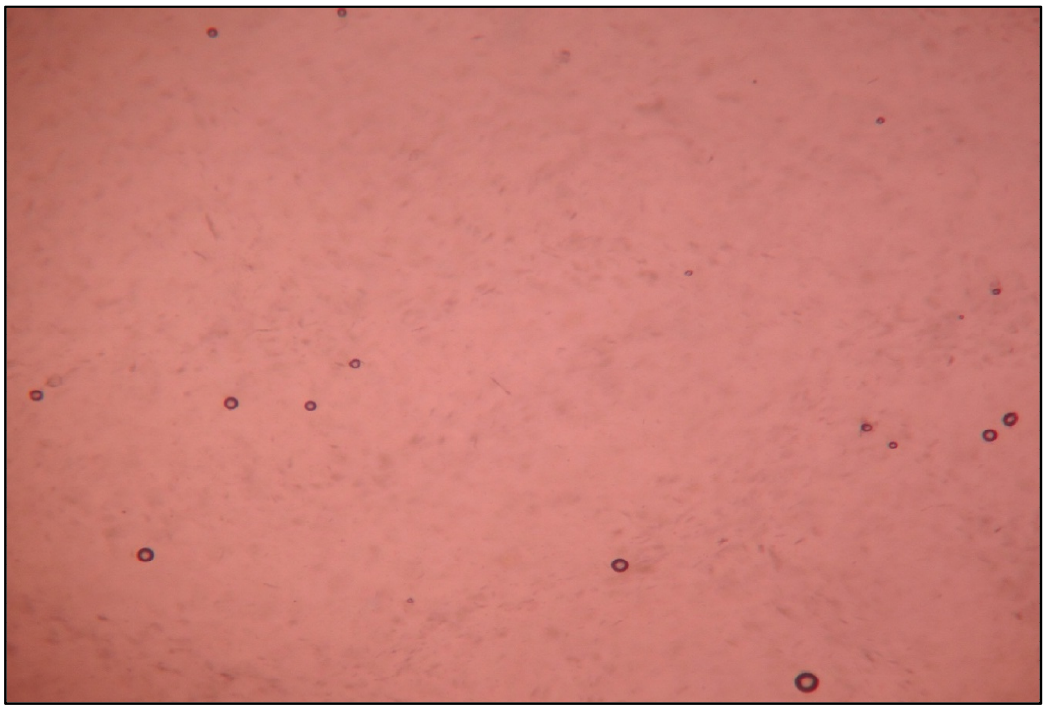


Fig no:5.9Microphotograph of groups of noisome at 45X Magnification

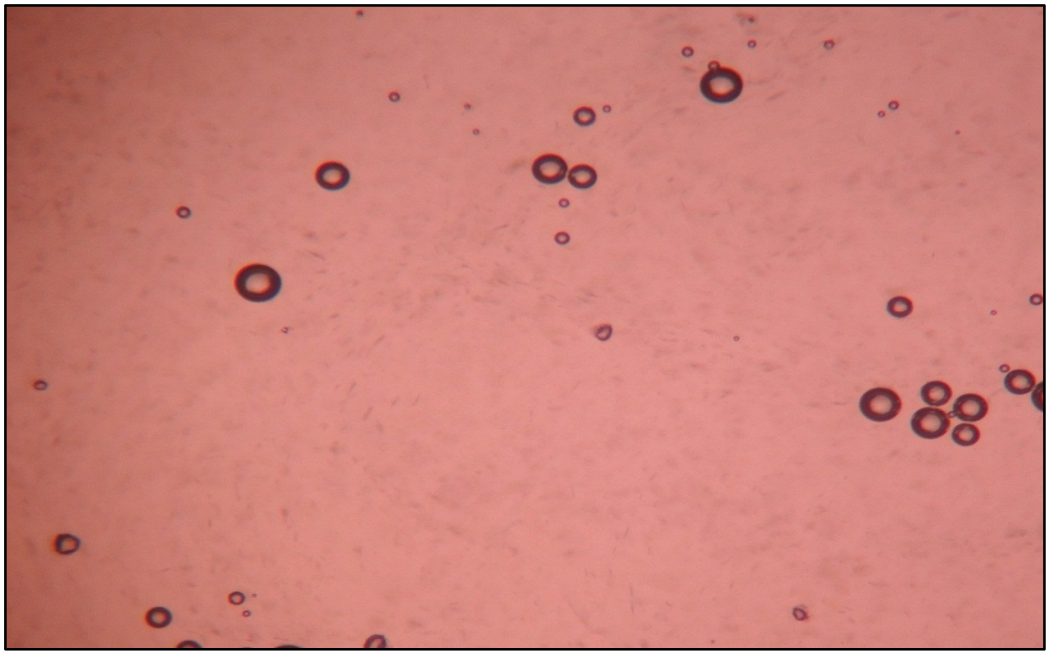


Fig no:5.10Microphoto graph of groups of noisome at 45X Magnification

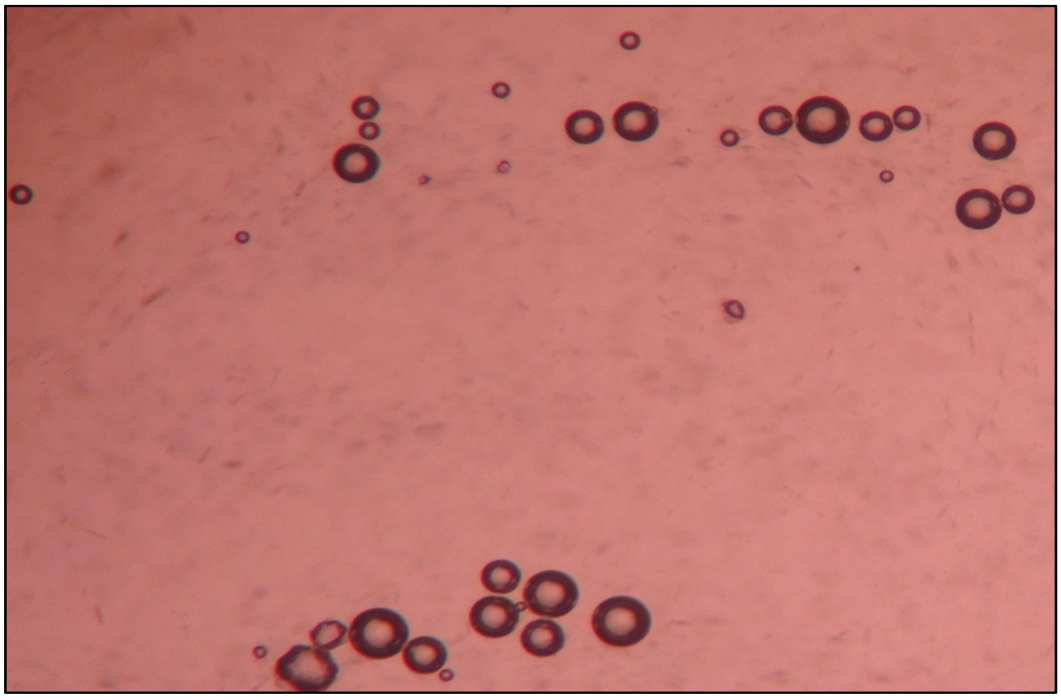


Fig no:5.11Transmission Electron Microscopic Image1

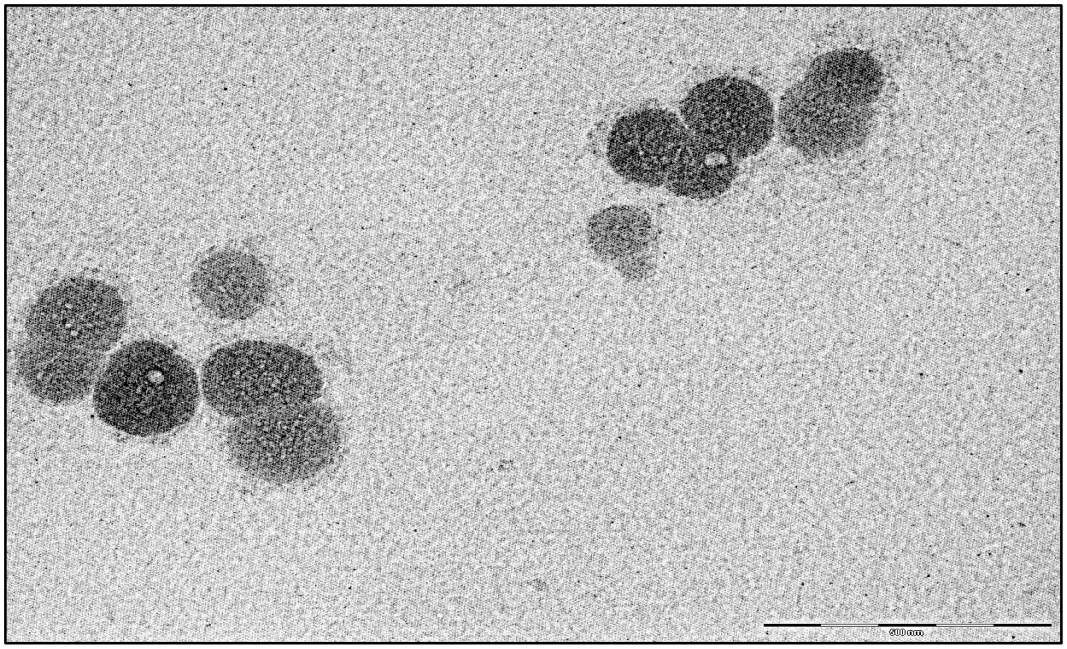


Fig no:5.12Transmission Electron Microscopic Image2

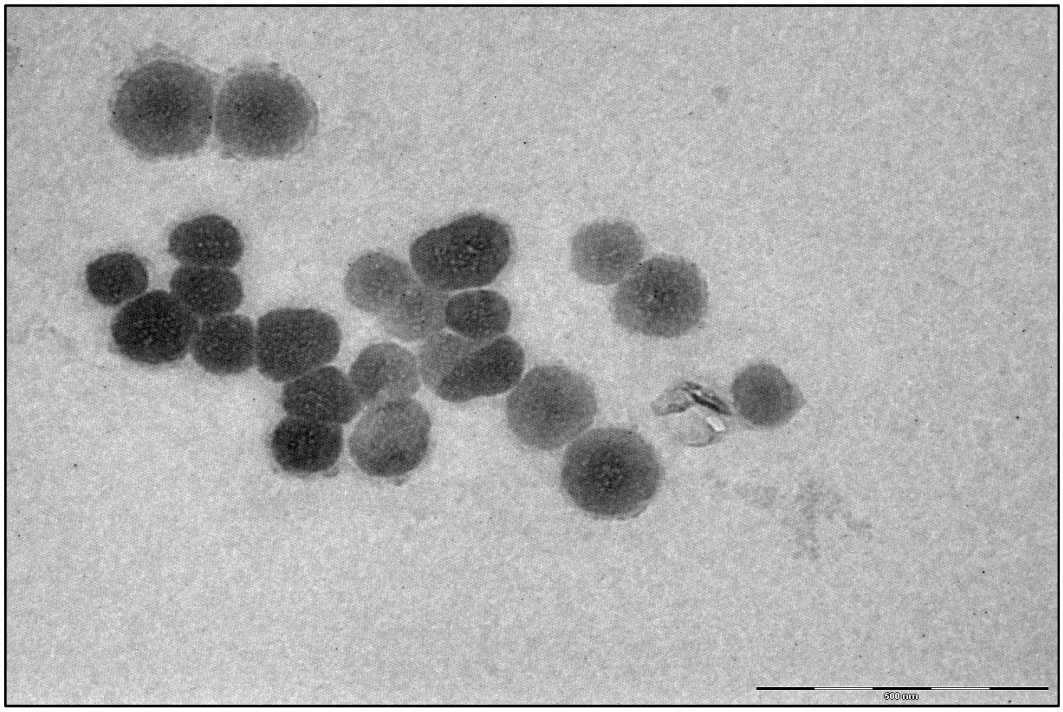
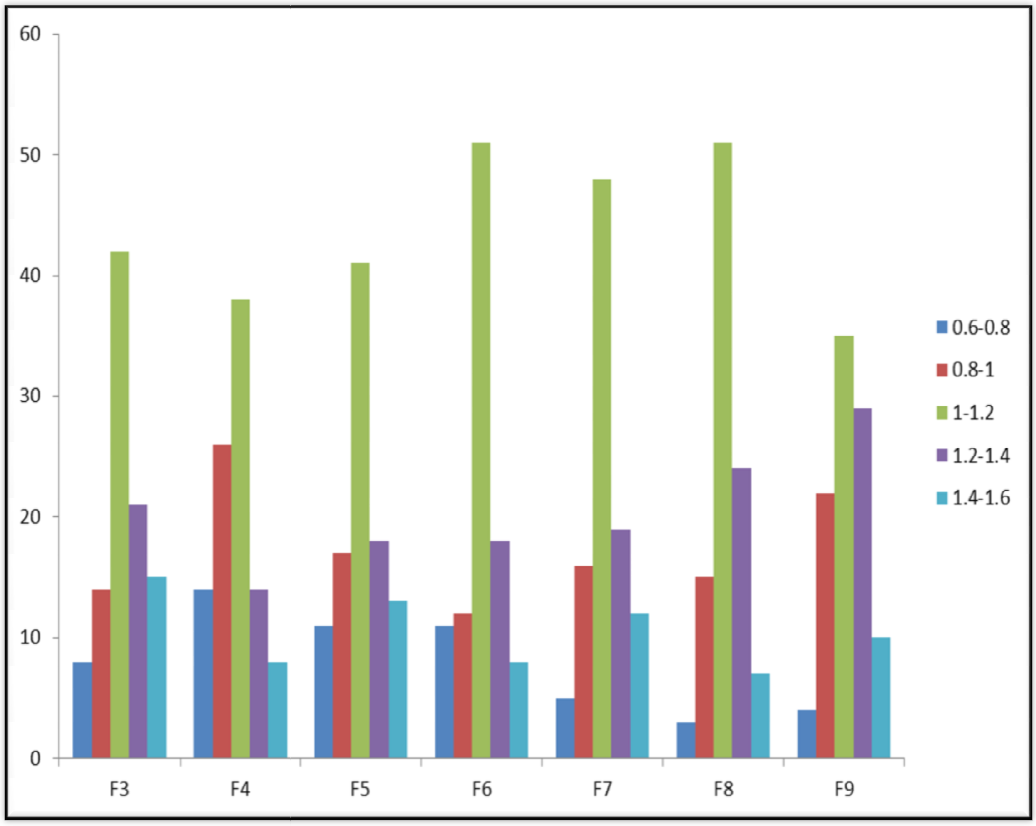


Table no:5.6 Particle size data of Bosentan monohydrate

SIZE RANGE(µm)	F3	F4	F5	F6	F7	F8	F9
0.6-0.8	8	14	11	11	5	3	4
0.8-1	14	26	17	12	16	15	22
1-1.2	42	38	41	51	48	51	35
1.2-1.4	21	14	18	18	19	24	29
1.4-1.6	15	8	13	8	12	7	10

Fig no:5.13 Graphical representation of Particle size distribution of Bosentan monohydrate



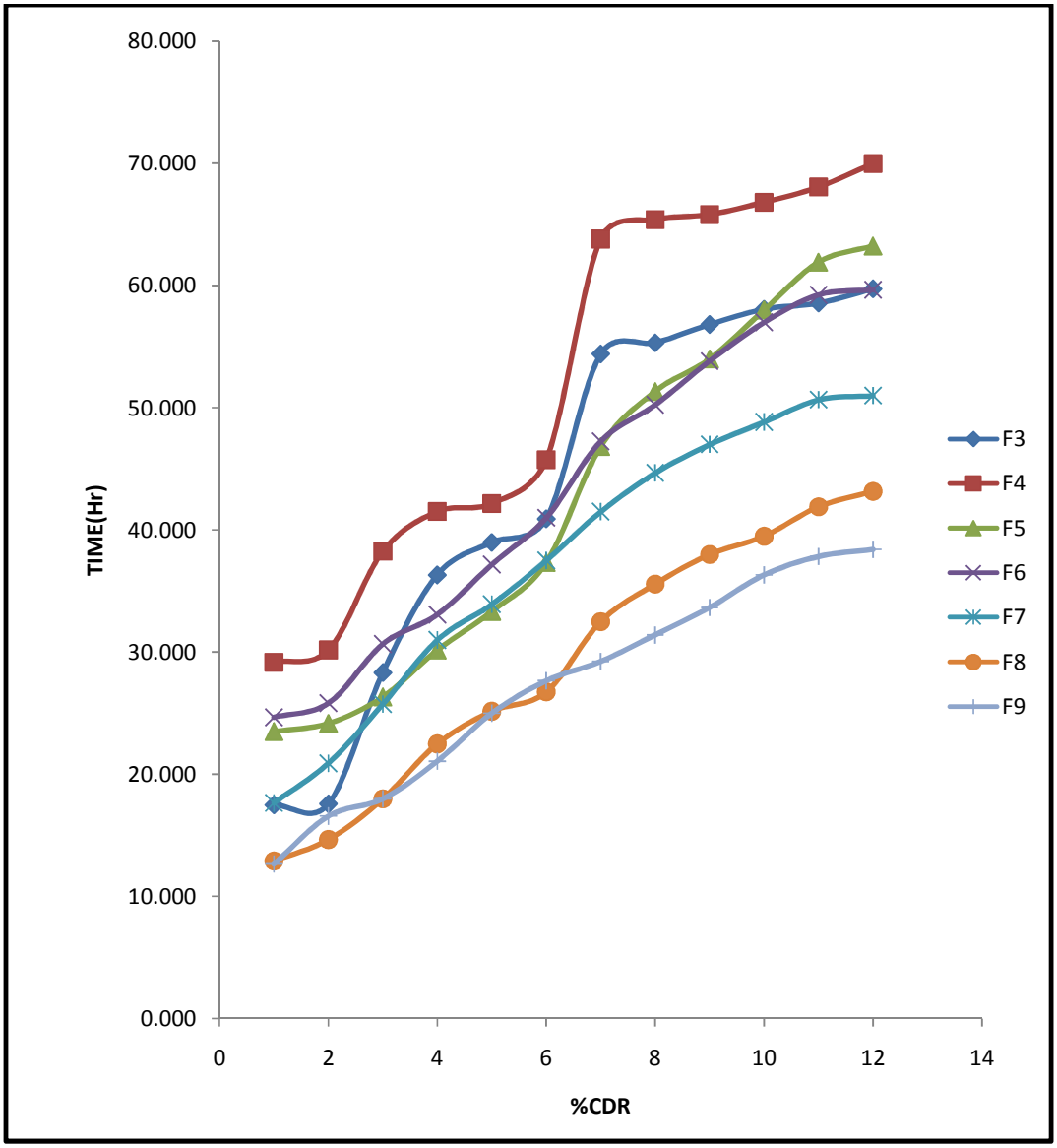
VI. Characterization of proniosomal suspension

A. In-vitro drug permeation study

Table no: 5.7% Cumulative drug release of Bosentan monohydrate in all formulations

TIME(Hr)	F3	F4	F5	F6	F7	F8	F9
1	17.500	29.167	23.500	24.667	17.667	12.917	12.667
2	17.583	30.167	24.167	25.833	20.917	14.667	16.583
3	28.333	38.250	26.333	30.667	25.750	18.000	18.000
4	36.333	41.500	30.167	33.083	31.000	22.500	21.083
5	39.000	42.167	33.333	37.167	33.917	25.167	25.000
6	40.917	45.750	37.333	41.000	37.500	26.750	27.667
7	54.417	63.833	46.833	47.250	41.500	32.500	29.250
8	55.333	65.417	51.333	50.250	44.667	35.583	31.417
9	56.833	65.833	54.000	53.833	47.000	38.000	33.667
10	58.083	66.833	58.000	57.000	48.833	39.500	36.333
11	58.583	68.083	61.917	59.250	50.667	41.917	37.833
12	59.750	70.000	63.250	59.667	51.000	43.167	38.417

Fig no:5.14 % Cumulative drug release curve of all formulations



B. **Release kinetics**

Table no:5.8 Release kinetic parameters of formulation F3

Time (hr)	Absorbance	Conc. (µg/ml)	% CDR	% DRM	SQRT Time (hr)	log % CDR	Log time	log % DRM
1	0.237	20.17	40.333	59.667	1.000	1.606	0.000	1.776
2	0.239	20.33	40.667	59.333	1.414	1.609	0.301	1.773
3	0.24	20.42	40.833	59.167	1.732	1.611	0.477	1.772
4	0.251	21.33	42.667	57.333	2.000	1.630	0.602	1.758
5	0.267	22.67	45.333	54.667	2.236	1.656	0.699	1.738
6	0.271	23.00	46.000	54.000	2.449	1.663	0.778	1.732
7	0.284	24.08	48.167	51.833	2.646	1.683	0.845	1.715
8	0.289	24.50	49.000	51.000	2.828	1.690	0.903	1.708
9	0.299	25.33	50.667	49.333	3.000	1.705	0.954	1.693
10	0.303	25.67	51.333	48.667	3.162	1.710	1.000	1.687
11	0.305	25.83	51.667	48.333	3.317	1.713	1.041	1.684
12	0.309	26.17	52.333	47.667	3.464	1.719	1.079	1.678

Table no:5.9 Release kinetic parameters of formulation F4

Time (hr)	Absorbance	Conc. (µg/ml)	% CDR	% DRM	SQRT Time (hr)	log % CDR	Log time	log % DRM
1	0.241	20.50	41.000	59.000	1.000	1.613	0.000	1.771
2	0.24	20.42	40.833	59.167	1.414	1.611	0.301	1.772
3	0.25	21.25	42.500	57.500	1.732	1.628	0.477	1.760
4	0.261	22.17	44.333	55.667	2.000	1.647	0.602	1.746
5	0.273	23.17	46.333	53.667	2.236	1.666	0.699	1.730
6	0.282	23.92	47.833	52.167	2.449	1.680	0.778	1.717
7	0.289	24.50	49.000	51.000	2.646	1.690	0.845	1.708
8	0.296	25.08	50.167	49.833	2.828	1.700	0.903	1.698
9	0.304	25.75	51.500	48.500	3.000	1.712	0.954	1.686
10	0.307	26.00	52.000	48.000	3.162	1.716	1.000	1.681
11	0.31	26.25	52.500	47.500	3.317	1.720	1.041	1.677
12	0.312	26.42	52.833	47.167	3.464	1.723	1.079	1.674

Table no:5.10 Release kinetic parameters of formulation F5

Time (hr)	Absorbance	Conc. (µg/ml)	% CDR	% DRM	SQRT Time (hr)	log % CDR	Log time	log % DRM
1	0.235	20.00	40.000	60.000	1.000	1.602	0.000	1.778
2	0.213	18.17	36.333	63.667	1.414	1.560	0.301	1.804
3	0.223	19.00	38.000	62.000	1.732	1.580	0.477	1.792
4	0.237	20.17	40.333	59.667	2.000	1.606	0.602	1.776
5	0.248	21.08	42.167	57.833	2.236	1.625	0.699	1.762
6	0.301	25.50	51.000	49.000	2.449	1.708	0.778	1.690
7	0.315	26.67	53.333	46.667	2.646	1.727	0.845	1.669
8	0.321	27.17	54.333	45.667	2.828	1.735	0.903	1.660
9	0.325	27.50	55.000	45.000	3.000	1.740	0.954	1.653
10	0.331	28.00	56.000	44.000	3.162	1.748	1.000	1.643
11	0.334	28.25	56.500	43.500	3.317	1.752	1.041	1.638
12	0.335	28.33	56.667	43.333	3.464	1.753	1.079	1.637

Table no:5.11 Release kinetic parameters of formulation F6

Time (hr)	Absorbance	Conc. (µg/ml)	% CDR	% DRM	SQRT Time (hr)	log % CDR	Log time	log % DRM
1	0.217	18.50	37.000	63.000	1.000	1.568	0.000	1.799
2	0.211	18.00	36.000	64.000	1.414	1.556	0.301	1.806
3	0.239	20.33	40.667	59.333	1.732	1.609	0.477	1.773
4	0.253	21.50	43.000	57.000	2.000	1.633	0.602	1.756
5	0.277	23.50	47.000	53.000	2.236	1.672	0.699	1.724
6	0.288	24.42	48.833	51.167	2.449	1.689	0.778	1.709
7	0.289	24.50	49.000	51.000	2.646	1.690	0.845	1.708
8	0.301	25.50	51.000	49.000	2.828	1.708	0.903	1.690
9	0.306	25.92	51.833	48.167	3.000	1.715	0.954	1.683
10	0.311	26.33	52.667	47.333	3.162	1.722	1.000	1.675
11	0.319	27.00	54.000	46.000	3.317	1.732	1.041	1.663
12	0.329	27.83	55.667	44.333	3.464	1.746	1.079	1.647

Table no:5.12 Release kinetic parameters of formulation F7

Time (hr)	Absorbance	Conc. (µg/ml)	% CDR	% DRM	SQRT Time (hr)	log % CDR	Log time	log % DRM
1	0.203	17.33	34.667	65.333	1.000	1.540	0.000	1.815
2	0.205	17.50	35.000	65.000	1.414	1.544	0.301	1.813
3	0.217	18.50	37.000	63.000	1.732	1.568	0.477	1.799
4	0.241	20.50	41.000	59.000	2.000	1.613	0.602	1.771
5	0.245	20.83	41.667	58.333	2.236	1.620	0.699	1.766
6	0.263	22.33	44.667	55.333	2.449	1.650	0.778	1.743
7	0.279	23.67	47.333	52.667	2.646	1.675	0.845	1.722
8	0.283	24.00	48.000	52.000	2.828	1.681	0.903	1.716
9	0.291	24.67	49.333	50.667	3.000	1.693	0.954	1.705
10	0.307	26.00	52.000	48.000	3.162	1.716	1.000	1.681
11	0.312	26.42	52.833	47.167	3.317	1.723	1.041	1.674
12	0.315	26.67	53.333	46.667	3.464	1.727	1.079	1.669

Table no:5.13 Release kinetic parameters of formulation F8

Time (hr)	Absorbance	Conc. (µg/ml)	% CDR	% DRM	SQRT Time (hr)	log % CDR	Log time	log % DRM
1	0.203	17.33	34.667	65.333	1.000	1.540	0.000	1.815
2	0.205	17.50	35.000	65.000	1.414	1.544	0.301	1.813
3	0.217	18.50	37.000	63.000	1.732	1.568	0.477	1.799
4	0.241	20.50	41.000	59.000	2.000	1.613	0.602	1.771
5	0.245	20.83	41.667	58.333	2.236	1.620	0.699	1.766
6	0.263	22.33	44.667	55.333	2.449	1.650	0.778	1.743
7	0.279	23.67	47.333	52.667	2.646	1.675	0.845	1.722
8	0.283	24.00	48.000	52.000	2.828	1.681	0.903	1.716
9	0.291	24.67	49.333	50.667	3.000	1.693	0.954	1.705
10	0.307	26.00	52.000	48.000	3.162	1.716	1.000	1.681
11	0.312	26.42	52.833	47.167	3.317	1.723	1.041	1.674
12	0.315	26.67	53.333	46.667	3.464	1.727	1.079	1.669

Table no:5.14 Release kinetic parameters of formulation F9

Time (hr)	Absorbance	Conc. (µg/ml)	% CDR	% DRM	SQRT Time (hr)	log % CDR	Log time	log % DRM
1	0.239	20.33	40.667	59.333	1.000	1.609	0.000	1.773
2	0.24	20.42	40.833	59.167	1.414	1.611	0.301	1.772
3	0.241	20.50	41.000	59.000	1.732	1.613	0.477	1.771
4	0.269	22.83	45.667	54.333	2.000	1.660	0.602	1.735
5	0.291	24.67	49.333	50.667	2.236	1.693	0.699	1.705
6	0.303	25.67	51.333	48.667	2.449	1.710	0.778	1.687
7	0.307	26.00	52.000	48.000	2.646	1.716	0.845	1.681
8	0.309	26.17	52.333	47.667	2.828	1.719	0.903	1.678
9	0.317	26.83	53.667	46.333	3.000	1.730	0.954	1.666
10	0.319	27.00	54.000	46.000	3.162	1.732	1.000	1.663
11	0.32	27.08	54.167	45.833	3.317	1.734	1.041	1.661
12	0.324	27.42	54.833	45.167	3.464	1.739	1.079	1.655

Fig no:5.15First order release kinetics of Bosentan monohydrate of formulationF3

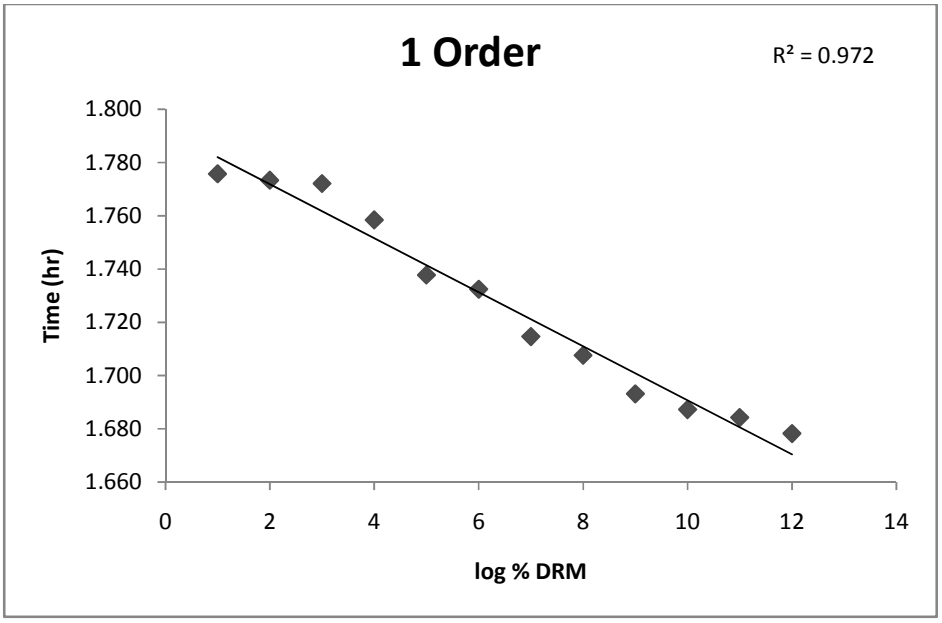


Fig no:5.16Firstorder release kinetics of Bosentan monohydrate of formulationF4

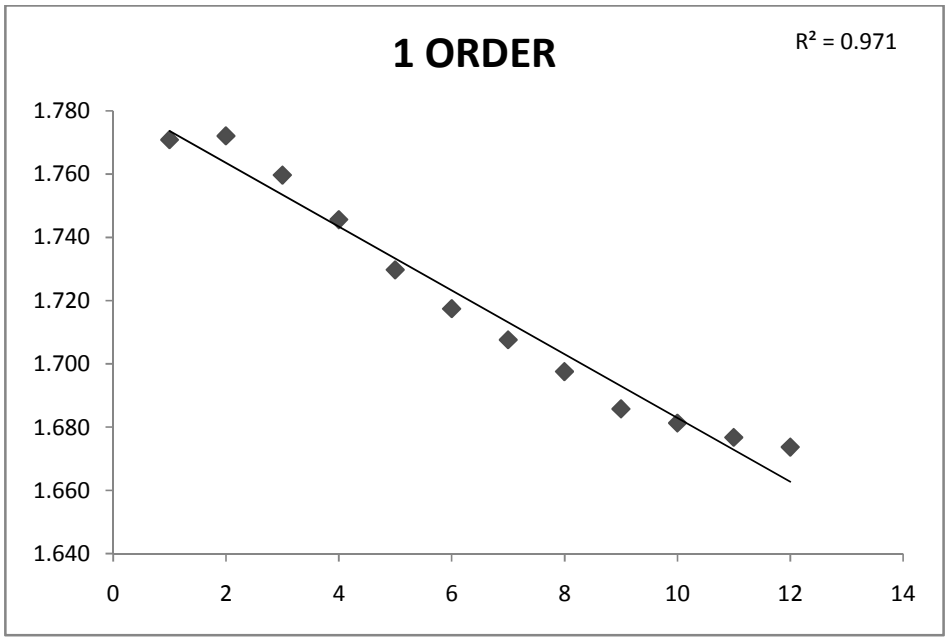


Fig no:5.17 First order release kinetics of Bosentan monohydrate of formulation F5

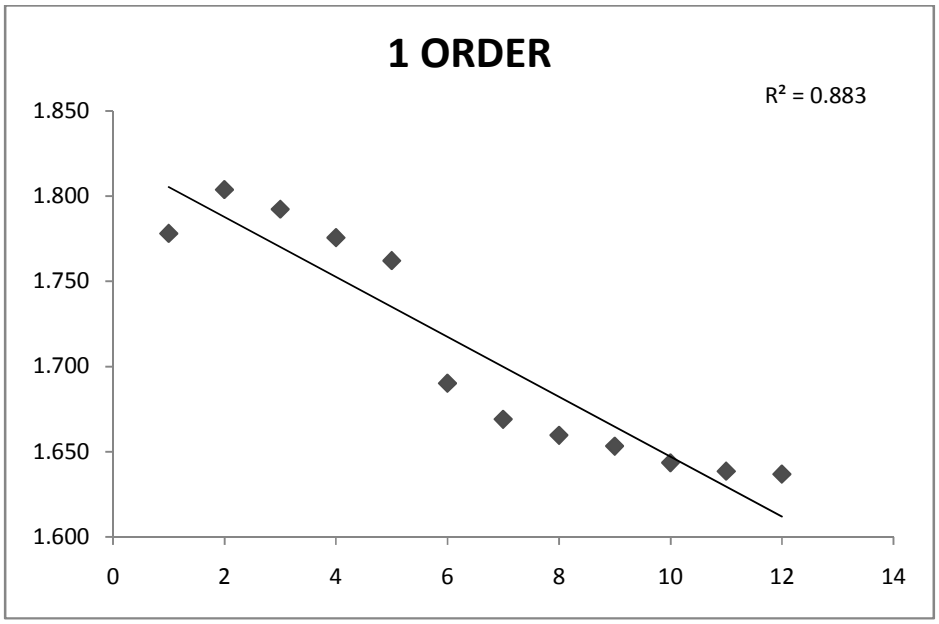


Fig no:5.18Higuchi release kinetics of Bosentan monohydrate of formulationF6

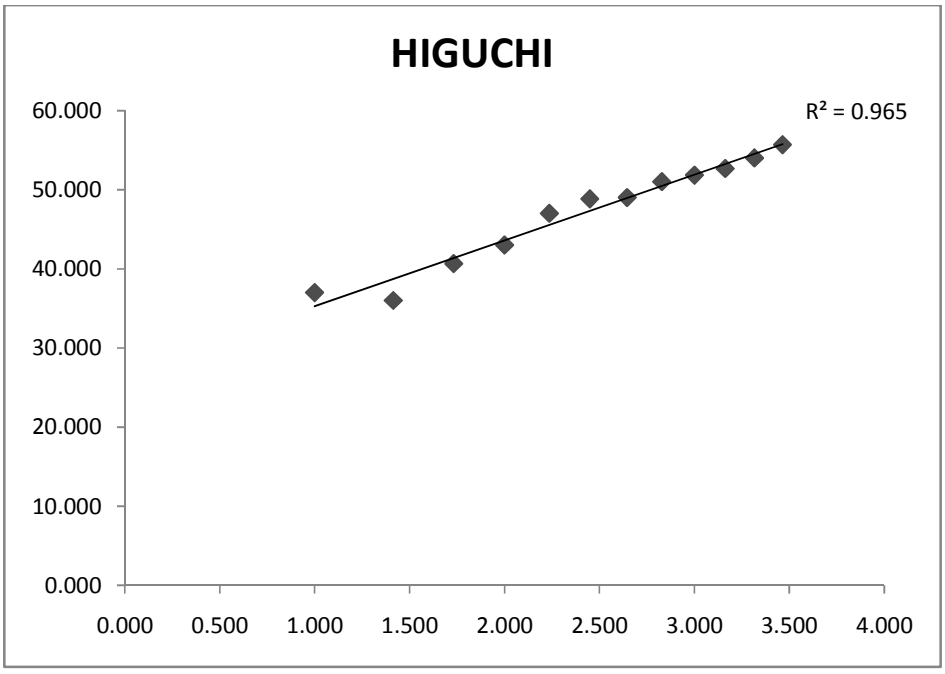


Fig no:5.19 First order release kinetics of Bosentan monohydrate of formulationF7

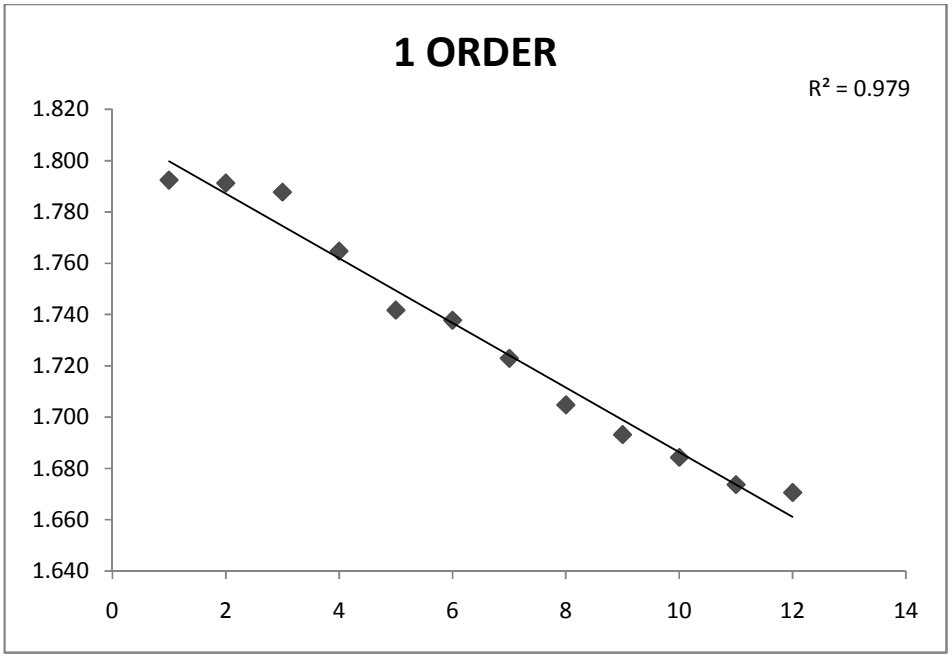


Fig no:5.20First order release kinetics of Bosentan monohydrate of formulationF8

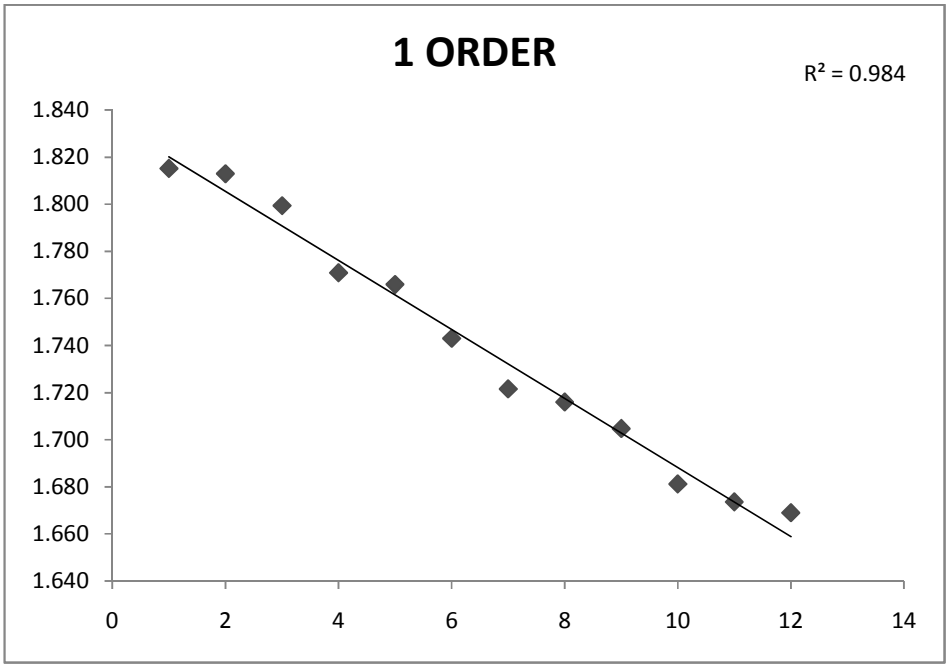


Fig no:5.21Higuchi release kinetics of Bosentan monohydrate of formulationF9

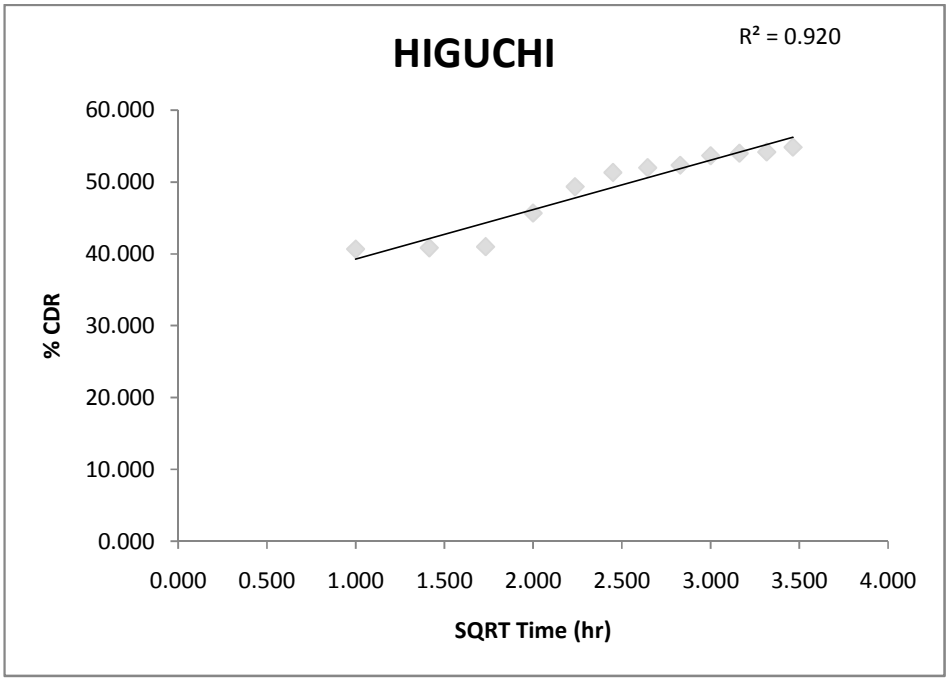


Table no:5.15Value of coefficient of regression (R^2) of all formulations

Formulation	R2 value				Best fit model
	Zero Order Model	First Order Model	Higuchi Model	Korsmeyer-Peppas Model	
F3	0.9687	0.9729	0.9541	0.8835	First order
F4	0.9648	0.9718	0.9716	0.9208	First order
F5	0.8711	0.8837	0.8562	0.7672	First order
F6	0.9397	0.9137	0.9656	0.9337	Higuchi model
F7	0.9731	0.9791	0.9621	0.8985	First order
F8	0.9777	0.9844	0.9748	0.9276	First order
F9	0.8813	0.8966	0.9209	0.8945	Higuchi model

DISCUSSION

Niosome are used to provide better penetration trough gastrointestinal tract, and thus provides better bioavailability. It also provides sustained release for drugs. Bosentan is used for treatment pulmonary hypertension. Bioavailability of Bosentan monohydrate was found to be increased in niosomal form as compare to other forms.

The Bosentan monohydrate identified by white crystalline powder, and bitter in taste, which is compliance with standard value of Bosentan monohydrate, its shows starting melting at 195ºC which was between the range, solubility of Bosentan monohydrate in different solvents was performed. The study indicate the affinity of Bosentan monohydrate towards non-aqueous solvents The solubility of Bosentan monohydrate was shown in several solvents and it was found to be soluble in some solvents like ethanol , methanol, acetone and dichloromethane and insoluble in some solvents as well like distilled water, n-hexane, pet ether etc.

The drug was identified by FT-IR spectra shown in (figure 8). FT-IR spectra of pure drug and drug with polymer were shown, no interaction was seen in both spectra. IR spectra of pure drug and its polymer used in formulations indicate that there were no structural changes caused by excipients.

The absorption maxima were determined by using UV/visible spectrophotometer found at 270nm which complies with standard value.In ethanol, absorbance was determined between ranges of 0.303 to 1.710. The determined absorbance shows linear absorption and value of the coefficient of regration was found to be $R^2 = 0.972$and equation of line was found to be $y = 0.0207x + 0.1441$. In phosphate buffer, absorbance was determined ranges “between” 0.039 to 0.619.calibration curve of Bosentan monohydrate show straight –line with coefficient

of regration was found to be R^2=0.9968 was found and equation of line was found to be y = 0.0129x - 0.0055

Out of different methods of preparation of noisome "formation of noisome from pro-niosome"was selected, cholesterol and span 20 were used in noisome formation. Different concentration of non-ionic surfactant was used for preparation of noisome

Temperature was maintained "between "55-60^{o}C". Below 40^{o}c temperature the noisome was not formed and above temperature 80.c the formulation changes in color before formation of pro-niosomal suspension.

Totally eleven formulations were formulated numbered from F1 to F11, out of which noisome were not observed as per our expectations in four formulations namely F1,F2, F10 and F11. So the first two and last two formulations were excluded from further evaluations and only the formulation from F3 to F9 were performed and been mentioned in the formulation chart.

Particle size analysis was performed by ocular light microscope the size range of the niosome was found between the ranges of 0.6-1.6 µm. The main factor affecting the size of noisome is cholesterol and HLB of non-ionic surfactant. Formulation F4 was found to have lesser particle size. Transmission electron microscopies shown noisome are spherical in shape

The entrapment efficiency was performed to estimate the actual amount of drug being entrapped. Maximum percent drug entrapment was seen in formulation F4 and minimum percent drug entrapment was seen in formulation F8..

Under perfect sink condition, the drug release rate depends on concentration of cholesterol and surfactant and drug release behavior of Bosentan monohydrate was studied in phosphate buffer (p^H 7.4) at 37±2^{O}c the curve was obtained after plotting cumulative amount of drug released from each formulation against time. Formulation F4 (70.00%) showed maximum release while other formulation showed less amount of drug release in 12 hrs. Formulation F4 follows drug release by first order model.

To predict the release pattern of Bosentan monohydrate from noisome formulation batches (F3 to F9) correlation coefficient and rate constant was calculated for zero order, first order, Higuchi order and Korsmeyer-Peppas Model kinetics. The studies of drug release kinetics showed that majority of the formulations are governed by first order kinetic model.

F4 formulation show maximum permeation flux of Bosentan monohydrate and the permeation of drug were enhanced by surfactant present in niosomes.

SUMMARY AND CONCLUSION

Summary

Niosomes or non-ionic surfactant vesicles are one of the many different carriers for transporting a drug molecule to its site of action. They are uni-lamellar or multi-lamellar vesicles where in an aqueous solution is enclosed in a highly ordered bilayer made up of non-ionic surfactants with or without cholesterol. They can entrap both hydrophilic and hydrophobic drugs. Niosomes are preferred over other vesicular system because of chemical stability, low toxicity, non-ionic nature, better bio-availability of drug at site, good intrinsic skin permeation and they are weakly immunogenic. Niosomes protect drugs against acidic and enzymatic degradation. Niosomes showed excellent entrapment efficiency and in-vitro drug release can be manipulated by the type of surfactant and type of its charge.

Formulation of Bosentan monohydrate was performed by using cholesterol, span 20, and ethanol. Preformulation study like solubility, melting pointand absorption maxima was performed and further IR was also performed which shows no interaction between drug and excipients used in the formulation the formulation was performed by the technique ”noisome formulation from proniosome”.

The Niosomes was characterized by entrapment efficiency that shows good entrapment of drug up to 85.198%.

Then the particle size of niosomes was analyzed using, transmission electron microscopewhich shows that the size is within the expected range and later it was confirmed by TEM analysis.

Ex-vivo drug permeation study was performed on freshly excised goat intestine and the study shows better bioavailability of Bosentan in the form of niosome.

Further the in-vitro release was performed and the release kinetics was foundand plotted for different modelsand found that maximum formulations follow the first order kinetics.

Conclusion

The present study demonstrated the successful preparation of Bosentan monohydrate niosomeby "noisome prepared from proniosome method" and their evaluation. Formulation F4 shows better bioavailability as well as sustained release as compare to other formulations. Based on the evaluations and the results the Bosentan monohydrate noisome containing span 20 and cholesterol be taken as an ideal formulation.

Table No-6

CALIBRATION CURVE OF RAMIPRIL IN PHOSPHATE BUFFERED SALINE pH 7.4.

S.No.	CONCENTRATION (µg/ml)	ABSORBANCE ± SD*
1	5	0.196 ± 0.0005
2	10	0.390 ± 0.007
3	15	0.587 ± 0.0057
4	20	0.781 ± 0.0065
5	25	0.974 ± 0.0045
6	30	1.182 ± 0.0091
7	35	1.382 ± 0.0043
8	40	1.564 ± 0.015
9	45	1.747 ± 0.016
10	50	1.972 ± 0.0087

n = 3*

γ = 0.999901195

Table No: 7
ENTRAPMENT EFFICIENCIES OF RAMIPRIL NIOSOMES USING SPAN 60: CHOLESTEROL (1:1) AT DIFFERENT MOLAR CONCENTRTIONS.

S.NO	MOLAR CONCENTRATION	% ENTARPMRNT EFFICIENCY ± SD*
1	20 µmol	32.28 ± 0.33
2.	30 µmol	35.30 ± 0.17
3.	40 µmol	34.11 ± 0.58
4.	50 µmol	30.62 ±0.69
5.	60 µmol	28.33 ± 0.47
6.	70 µmol	25.94 ± 0.28

n=3*

Table No: 8
FORMULATION OF RAMIPRIL NIOSOMES

S.NO	FORMULATION	SURFACTANT	RATIO OF	
			SURFACTANT	CHOLESTEROL
1.	F1	SPAN 60	1	1
2.	F2	SPAN 60	2	1
3.	F3	SPAN 60	3	1
4.	F4	SPAN 60	4	1
5.	F5	SPAN 60	5	1
6.	F6	SPAN 60	6	1
7.	F7	SPAN 20	1	1
8.	F8	SPAN 40	1	1
9.	F9	SPAN 80	1	1
10.	F10	TWEEN 60	1	1

Drug concentration used in each formulation kept as constant 2.5mg/5ml.

In ratio 1 stands for 30µmol.

Table No: 9

% ENTRAPMENT EFFICIENCY OF DIFFERENT FORMULATIONS

S.NO	FORMULATION	SURFACTANT	RATIO OF		% ENTRAPMENT EFFICIENCY ± SD*
			SURFACTANT	CHOLESTEROL	
1.	F1	SPAN 60	1	1	35.05±0.46
2.	F2	SPAN 60	2	1	30.76±0.65
3.	F3	SPAN 60	3	1	29.16±0.56
4.	F4	SPAN 60	4	1	28.06±0.71
5.	F5	SPAN 60	5	1	27.57±0.39
6.	F6	SPAN 60	6	1	25.28±0.50
7.	F7	SPAN 20	1	1	29.42±0.57
8.	F8	SPAN 40	1	1	33.54±0.65
9.	F9	SPAN 80	1	1	26.05±0.70
10.	F10	TWEEN 60	1	1	22.05±0.58

n=3*

Table No: 10

COMPARISON OF INVITRO RELEASE PROFILE OF RAMIPRIL NIOSOMES

TIME IN HOURS	CUMULATIVE % DRUG RELEASE ± SD*				
	F1 (SPAN 60 1:1)	F2 (SPAN 60 1:2)	F3 (SPAN 60 1:3)	F4 (SPAN 60 1:4)	F5 (SPAN 60 1:5)
0.00	0 ±0	0 ±0	0 ±0	0 ±0	0 ±0
0.25	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
0.5	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
0.75	0 ± 0	3.7 ± 1.80	4.7 ± 0.81	4.8 ± 0.51	5.5 ± 0 .61
1.0	4.1 ± 0.24	7.4 ± 1.09	8.2 ± 1.02	7.7 ± 0.99	8.6 ± 1.32
1.5	7.7 ± 1.71	11.5 ± 0.87	12.3 ± 0.98	13.2 ± 0.69	13.3 ± 1.00
2.0	11.3 ± 1.75	15.7 ± 0.96	16.1 ± 1.84	17.6 ± 1.59	18.4 ± 1.00
2.5	15.9 ± 1.67	19.6 ± 1.46	21.4 ± 1.51	22.6 ± 2.09	25.5 ± 1.49
3.0	19.7 ± 2.54	23.8 ± 1.07	24.3 ± 2.03	26.8 ± 1.88	30.9 ± 1.48
3.5	24.5 ± 2.01	26.5 ± 2.48	29.8 ± 1.42	30.0 ± 2.00	34.9 ± 1.79
4.0	26.7 ± 1.76	29.7 ± 0.75	32.1 ± 1.52	31.7 ± 1.45	39.3 ± 2.20
4.5	29.3 ± 1.10	31.7 ± 1.59	35.5 ± 1.70	34.1 ± 0.70	42.6 ± 1.29
5.0	31.4 ± 1.95	37.0 ±1.62	38.3 ± 1.65	36.1 ± 1.29	44.1 ± 0.89
5.5	33.9 ± 0.60	39.7 ± 0.40	41.1 ± 0.96	40.7 ± 1.29	47.8 ± 1.29
6.0	34.8 ± 0.40	42.6 ± 1.49	42.6 ± 0.47	45.6 ± 1.30	51.0 ±2.09
6.5	37.2 ± 1.04	46.6 ± 1.85	46.0 ± 1.18	49.6 ± 1.30	56.5 ± 0.52
7.0	39.2 ± 0.94	48.6 ± 1.34	50.2 ± 1.07	52.3 ± 0.65	56.9 ± 0.22
7.5	42.6 ± 0.58	50.8 ± 1.17	53.7 ± 0.94	57.0 ± 1.97	60.8 ± 1.89
8.0	44.0 ± 1.16	53.1 ± 1.17	56.0 ± 1.95	61.4 ± 0.83	64.1 ± 0.52
9.0	46.5 ± 1.28	54.7 ± 1.60	58.4 ± 0.96	65.8 ± 0.95	68.1 ± 1.29
10.0	49.7 ± 1.19	59.1 ± 1.10	61.1 ± 1.15	67.0 ± 0.19	71.4 ± 2.39
11.0	52.1 ± 0.02	60.7 ± 1.00	63.2 ± 1.98	69.6 ± 1.05	74.9 ± 1.39
12.0	54.5 ± 0.65	63.8 ± 1.50	66.7 ± 1.57	70.8 ± 1.39	79.9 ± 0.70

n=3*

Table No: 11

COMPARISON OF INVITRO RELEASE OF RAMIPRIL NIOSOMES

TIME IN HOURS	CUMULATIVE % DRUG RELLEASE ± SD*				
	F6(SPAN 60 1:6)	F7(SPAN 20 1:1)	F8(SPAN 40 1:1)	F9(SPAN80 1:1)	F10(TWEEN60 1:1)
0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
0.25	0 ± 0	5.0 ± 0.42	4 ± 0.51	4.1 ± 0.80	4.6 ±0.22
0.5	0 ±0	7.0 ± 0.69	5.9 ± 1.51	6.2 ± 1.18	6.8 ±1.49
0.75	5.2 ±0 .99	10.6 ± 1.39	8.5 ± 1.16	8.2 ± 2.30	9.3 ±1.03
1.0	8.3 ± 1.29	15.3 ± 1.14	11.6 ± 1.14	10.9 ± 1.65	14.0 ±1.09
1.5	11.4 ± 0.59	19.5 ± 1.69	14.0 ± 1.72	15.0 ± 1.25	18.8 ±2.50
2.0	14.6 ± 1.59	23.8 ± 0.59	17.0 ± 1.17	18.1 ± 1.53	23.3 ±1.34
2.5	18.5 ± 1.69	28.4 ± 0.99	19.7 ± 1.16	21.0 ± 1.26	28.2 ±1.28
3.0	22.3 ± 1.99	33.2 ± 0.71	23.7 ± 1.19	23.6 ± 1.98	32.0 ±1.29
3.5	25.6 ± 2.39	36.5 ± 1.30	26.7 ± 1.00	26.0 ± 1.26	36.2 ±1.19
4.0	28.9 ± 1.42	39.1 ± 1.32	30.0 ± 1.20	29.2 ± 1.04	40.8 ±1.34
4.5	32.2 ± 2.21	42.2 ± 0.61	32.4 ± 1.23	32.1 ± 1.03	45.5 ±1.92
5.0	35.9 ± 3.89	44.5 ± 0.91	34.3 ± 1.19	35.6 ± 2.01	49.9 ±1.27
5.5	39.4 ± 2.89	48.7 ± 1.80	38.0 ± 1.15	38.7 ± 1.76	54.5 ±1.08
6.0	42.1 ± 1.51	51.1 ± 1.48	40.5 ± 1.13	40.9 ± 1.26	57.7 ±1.25
6.5	46.2 ± 1.21	53.9 ± 1.23	43.9 ± 1.86	43.8 ± 1.25	61.2 ±1.20
7.0	48.9 ± 1.41	57.4 ± 2.01	46.8 ± 1.26	46.7 ±1.20	65.6 ±0.74
7.5	51.8 ± 0.71	59.9 ± 1.74	49.5 ± 0.01	48.8 ±1.92	70.3 ±1.79
8.0	53.7 ± 0.80	62.8 ± 0.94	51.5 ± 1.24	50.9 ±1.95	74.6 ±1.02
9.0	56.4 ± 1.10	67.4 ± 1.17	52.7 ± 0.25	52.6 ±1.17	79.5 ±1.09
10.0	58.9 ± 1.31	70.4 ± 2.11	55.5 ± 0.92	55.8 ±1.10	84.0 ±1.03
11.0	62.7 ± 1.69	73.9 ± 1.16	58.8 ± 1.62	58.2 ±1.49	88.2 ±1.04
12.0	65.4 ± 0.59	77.4 ± 1.18	61.4 ± 0.74	62.2 ±1.08	94.4 ±1.04

n=3*

Table No: 12
COMPARISON ON INVITRO RELEASE OF RAMIPRIL NIOSOMES (SPAN 60 1:1) WITH RAMIPRIL DRUG SOLUTION

TIME IN HOURS	CUMULATIVE % DRUG RELEASE±SD*	
	F1 (SPAN 60 1:1)	PURE DRUG SOLUTION
0	0 ± 0	0 ± 0
0.25	0 ± 0	30.3 ± 0.83
0.5	0 ± 0	43.3 ± 1.40
0.75	0 ± 0	48.6 ± 1.31
1.0	4.1 ± 0.24	54.9 ± 0.70
1.5	7.7 ± 1.71	62.3 ± 1.69
2.0	11.3 ± 1.75	68.1 ± 1.59
2.5	15.9 ± 1.67	75.7 ± 1.49
3.0	19.7 ± 2.54	85.8 ± 2.96
3.5	24.5 ± 2.01	96.4 ± 2.78
4.0	26.7 ± 1.76	97.3 ± 2.67
4.5	29.3 ± 1.10	98.0 ± 1.08
5.0	31.4 ± 1.95	98.7 ± 1.05
5.5	33.9 ± 0.60	98.6 ± 1.13
6.0	34.8 ± 0.40	97.6 ± 0.79
6.5	37.2 ± 1.04	97.3 ± 0.45
7.0	39.2 ± 0.94	97.3 ± 0.67
7.5	42.6 ± 0.58	97.2 ± 1.56
8.0	44.0 ± 1.16	97.1 ± 0.57
9.0	46.5 ± 1.28	96.6 ± 0.94
10.0	49.7 ± 1.19	96.3 ± 1.47
11.0	52.1 ± 0.02	96.0 ± 1.18
12.0	54.5 ± 0.65	95.7 ± 0.60

n=3*

Table no: 13
EFFECT OF SONICATION TIME (SPAN 60 1:1)

S.NO	SONICATION TIME (MINUTES)	% ENTRAPMENT EFFICIENCY
1	0	35.90
2	1	38.64
3	2	38.92
4	3	47.22
5	4	42.51
6	5	36.54

Table no: 14
EFFECT OF HYDRATION TIME (SPAN 60 1:1)

S.NO	HYDRATION TIME(MINUTES)	% ENTRAPMENT EFFICIENCY
1	30	35.22
2	45	52.21
3	60	41.63
4	75	31.04
5	90	13.12

Table No: 15

FORMULATION OF RAMIPRIL NIOSOMES WITH AND WITHOUT CHARGE INDUCING AGENTS.

S.NO.	FORMULATION	SURFACTANT : CHOLESTEROL (1:1 MOLAR RATIO)	CHARGE INDUCING AGENTS	
			STR	DCP
1.	F1	30 µmol	--	--
2.	F11	30 µmol	5 µmol	--
3.	F12	30 µmol	--	10 µmol
4.	F13	30 µmol	--	5 µmol
5.	F14	30 µmol	--	15 µmol

Drug concentration used in each formulation kept as constant 2.5mg/5ml.

Table No: 16

ENTRAPMENT EFFICIENCIES OF RAMIPRIL NIOSOMES WITH AND WITHOUT CHARGE INDUCING AGENTS.

S.NO	FORMULATION	% ENTRAPMENT EFFICIENCY ± SD*
1.	F1	35.05±0.46
2.	F11	40.53±0.33
3.	F12	27.88±0.48
4.	F13	20.45±0.88
5.	F14	20.62±0.56

n=3*

Table No: 17

COMPARISON ON INVITRO RELEASE OF RAMIPRIL NIOSOMES (SPAN 60 1:1) WITH AND WITHOUT CHARGE INDUCING AGENTS.

TIME IN HOURS	CUMULATIVE % DRUG RELEASE±SD*		
	F1	F11(STR)	F12(DCP)
0	0 ± 0	0 ± 0	0 ± 0
0.25	0 ± 0	1.10 ± 0.67	1.30 ± 1.51
0.5	0 ± 0	2.30 ± 0.96	2.60 ± 1.21
0.75	0 ± 0	3.90 ± 1.80	3.90 ± 0.80
1.0	4.1 ± 0.24	5.50 ± 1.09	8.20± 1.02
1.5	7.7 ± 1.71	8.30 ± 0.87	11.6 ± 0.98
2.0	11.3 ± 1.75	12.2 ± 0.96	15.8 ± 1.80
2.5	15.9 ± 1.67	14.6 ± 1.46	20.9 ± 1.53
3.0	19.7 ± 2.54	17.3 ± 1.07	23.8 ± 2.02
3.5	24.5 ± 2.01	19.5 ± 2.48	28.5 ± 1.40
4.0	26.7 ± 1.76	20.5 ± 0.75	33.4 ± 1.54
4.5	29.3 ± 1.10	22.6 ± 1.60	36.0 ± 1.72
5.0	31.4 ± 1.95	24.5 ± 1.60	39.0 ± 1.65
5.5	33.9 ± 0.60	27.8 ± 0.40	40.5 ± 0.96
6.0	34.8 ± 0.40	32.1 ± 1.50	42.6 ± 0.47
6.5	37.2 ± 1.04	35.7 ± 1.85	45.7 ± 1.18
7.0	39.2 ± 0.94	39.2 ± 1.34	49.8 ± 1.07
7.5	42.6 ± 0.58	42.2 ± 1.17	52.8 ± 0.94
8.0	44.0 ± 1.16	45.4 ± 1.00	56.8 ± 1.95
9.0	46.5 ± 1.28	49.0 ± 1.60	59.0 ± 0.96
10.0	49.7 ± 1.19	52.8 ± 1.10	60.3 ± 1.15
11.0	52.1 ± 0.02	55.3 ± 1.00	63.2 ± 1.98
12.0	54.5 ± 0.65	58.5 ± 1.50	67.4 ± 1.57

n=3* STR- Stearylamine DCP- Dicetyl phosphate.

Table No: 18

DETERMINATTION OF ORDER OF RELEASE OF RAMIPRIL FROM NIOSOMAL FORMULATIONS

Formulation	Higuchi	Korsemeyer-Peppas		Zero order		First order		Hixson-Crowell		Release mechanism
	r^2	r^2	n	r^2	K_0 (% mg/h)	r^2	K_1 (h^{-1})	r^2	Slope (n)	
F1	0.9945	0.9581	0.9853	0.957	4.997	0.9885	0.0306	0.980	0.0968	NFD
F2	0.9926	0.9697	0.9533	0.960	5.800	0.9926	0.0392	0.986	0.1262	NFD
F3	0.9945	0.9777	0.9108	0.959	6.007	0.9938	0.0418	0.986	0.1196	NFD
F4	0.9869	0.9796	0.9369	0.968	6.559	0.9882	0.0487	0.987	0.1437	NFD
F5	0.9969	0.9749	0.9301	0.961	7.066	0.9971	0.0572	0.995	0.1625	NFD
F6	0.9917	0.9906	0.9066	0.969	5.921	0.9954	0.0405	0.991	0.1231	NFD
F7	0.9974	0.9939	0.7280	0.963	6.401	0.9984	0.0522	0.996	0.1067	NFD
F8	0.9897	0.9967	0.7341	0.968	5.206	0.9935	0.0350	0.988	0.1067	NFD
F9	0.9916	0.9977	0.7284	0.969	5.188	0.9951	0.0350	0.988	0.1071	NFD
F10	0.9876	0.9972	0.8157	0.984	8.003	0.9409	0.0876	0.988	0.2196	NFD

NFD- NONFICKIAN DIFFUSION

Table no: 19

STABILITY STUDIES OF NIOSOMES CONTAINING SPAN 60 (1:1)

S.NO	WEEK	% ENTRAPMENT EFFICIENCY AT 60% RH±5% RH	
		4 ± 2^0C	25 ± 2^0 C
1.	0	35.05	35.05
2.	1	35.00	34.54
3.	2	34.88	33.21
4.	3	34.29	32.44
5.	4	33.81	31.07

Figure: 10

DETERMINATION OF λ_{max} OF RAMIPRIL IN PHOSPHATE BUFFERED SALINE PH- 7.4.

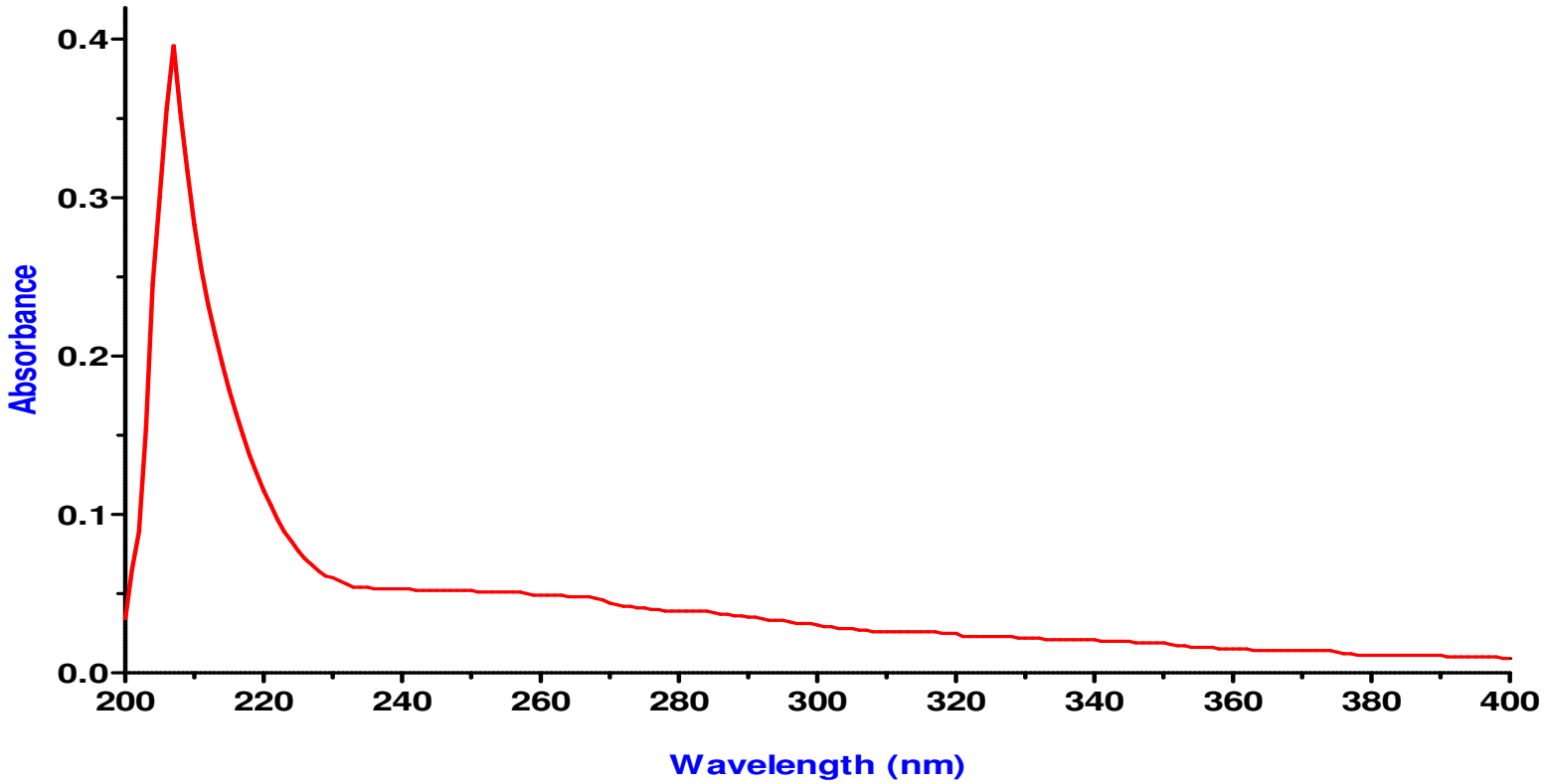


Figure- 11

CALIBRATION OF RAMIPRIL IN PBS (pH 7.4) AT λ_{max} 207nm

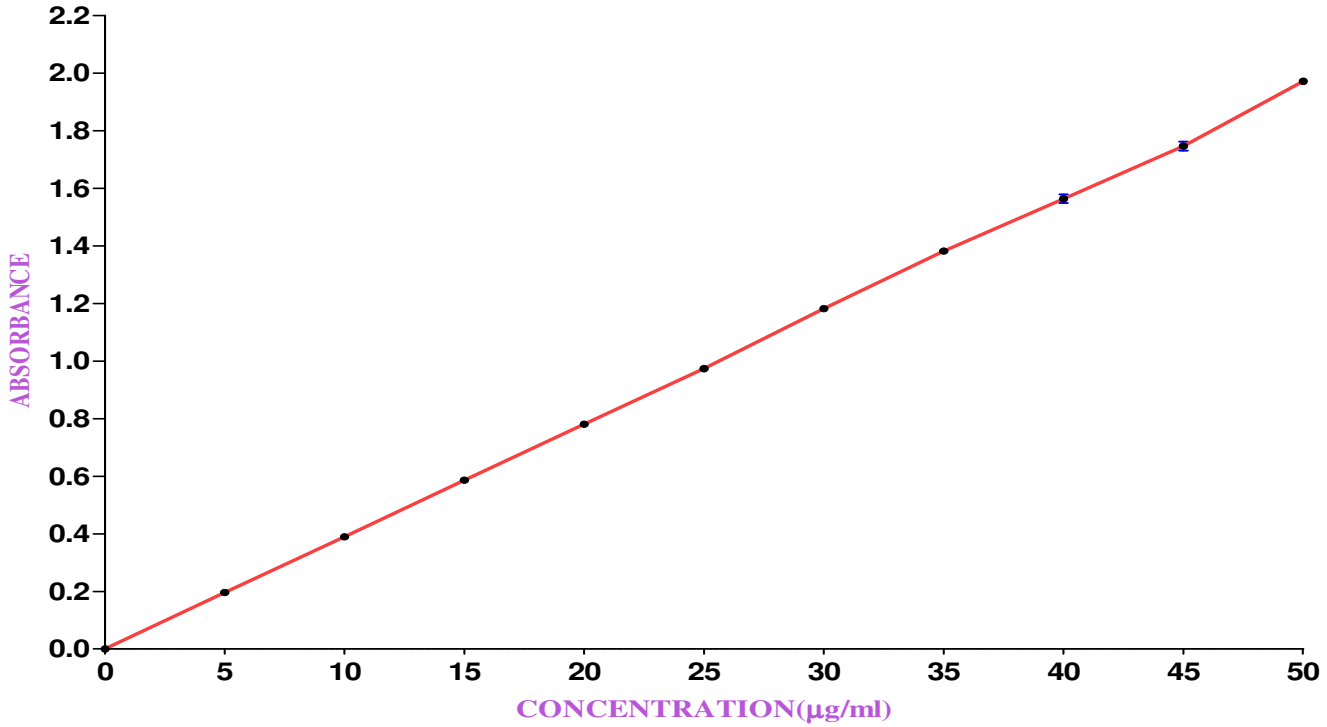


Figure- 12

COMPARISON OF ENTRAPMENT EFFICIENCIES OF RAMIPRIL NIOSOMES.

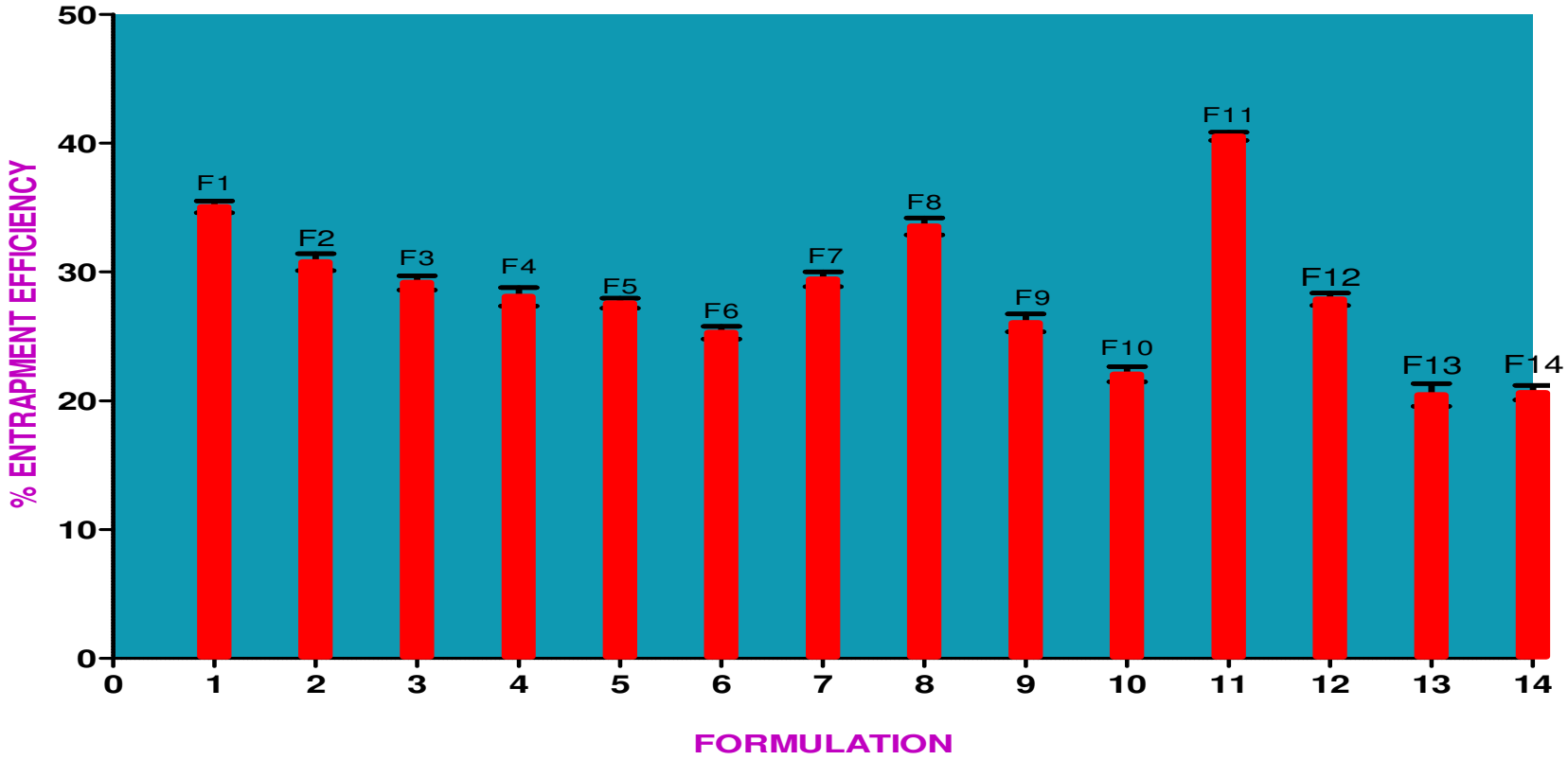


Figure- 13

COMPARISON OF INVITRO RELEASE OF RAMIPRIL NIOSOMES CONTAINING SPAN 60 AT DIFFERENT RATIOS.

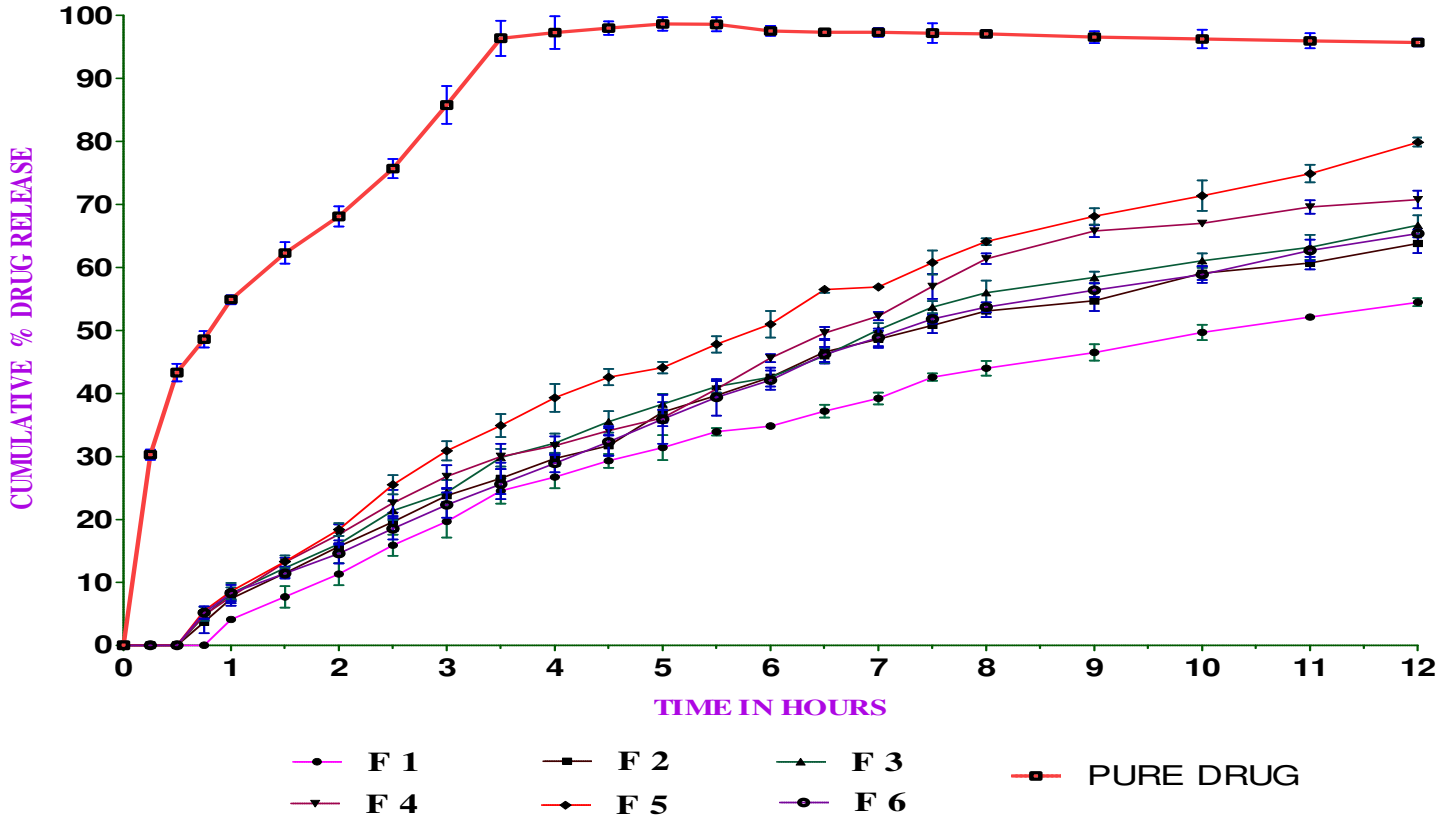


Figure- 14

COMPARISON OF INVITRO RELEASE OF RAMIPRIL NIOSOMES CONTAINING DIFFERENT SURFACTANTS AT 1:1 RATIO.

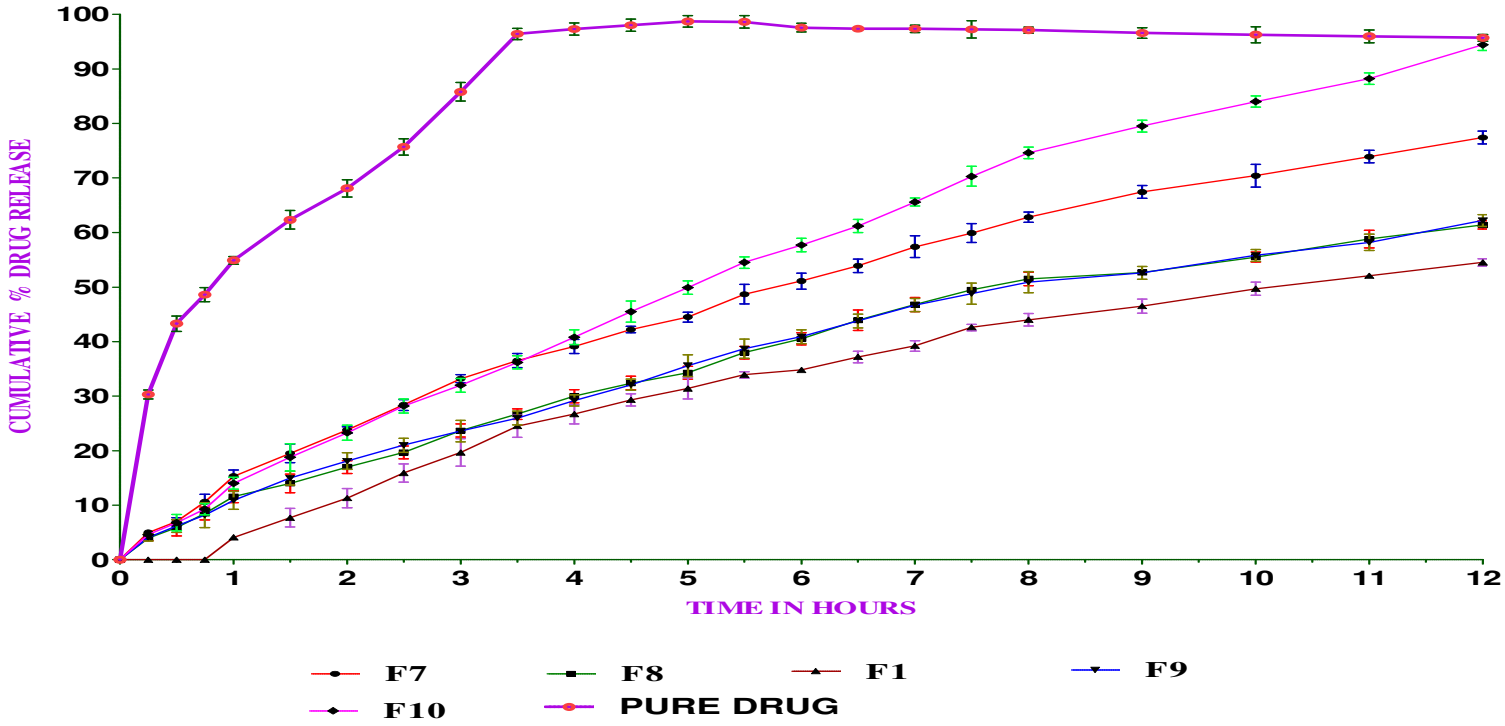


Figure: 15 a.

PARTICLE SIZE DISTRIBUTION F1

(SONICATION TIME= 0 MINUTES)

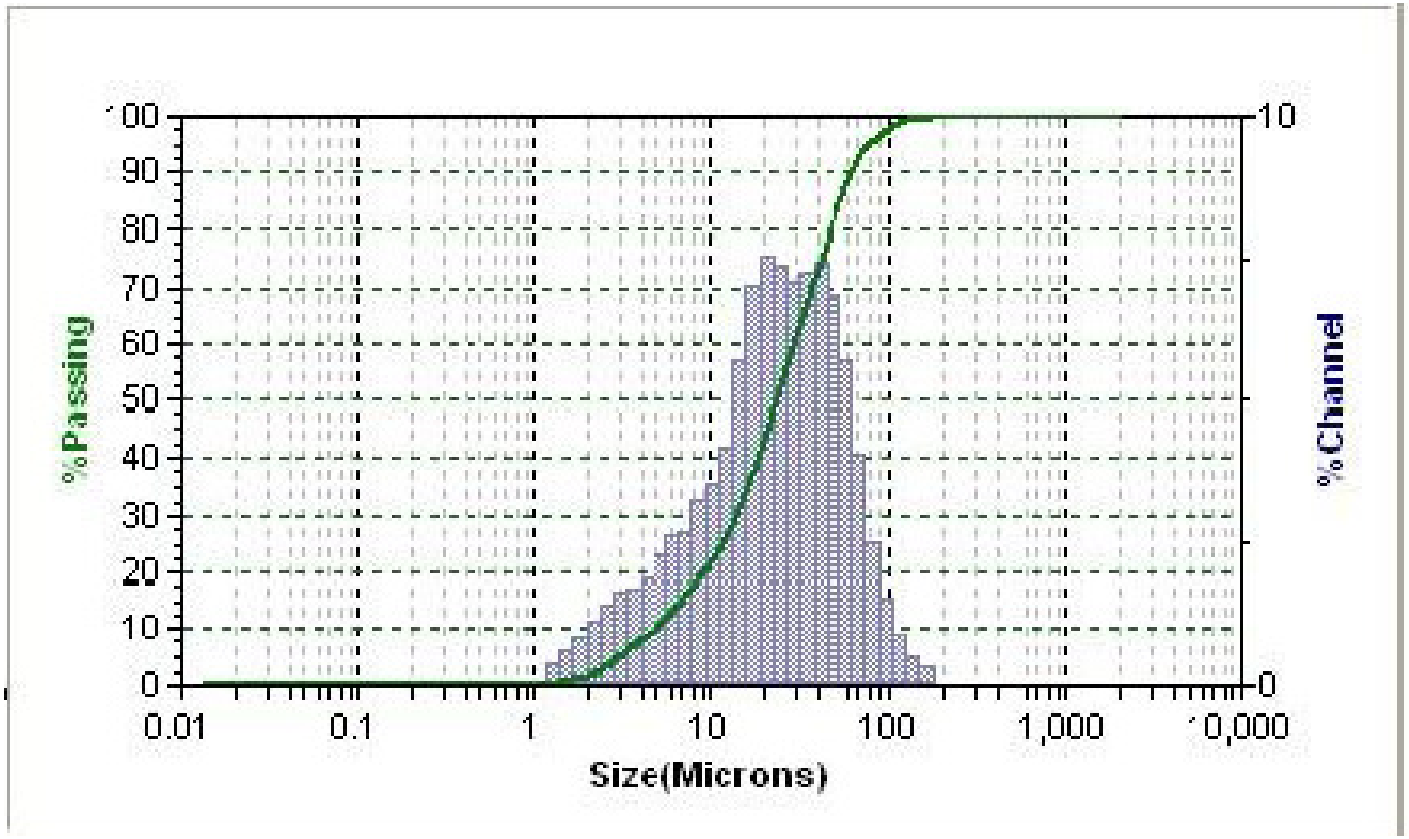


Figure: 15 b.

PARTICLE SIZE DISTRIBUTION F1

(SONICATION TIME= 1 MINUTE)

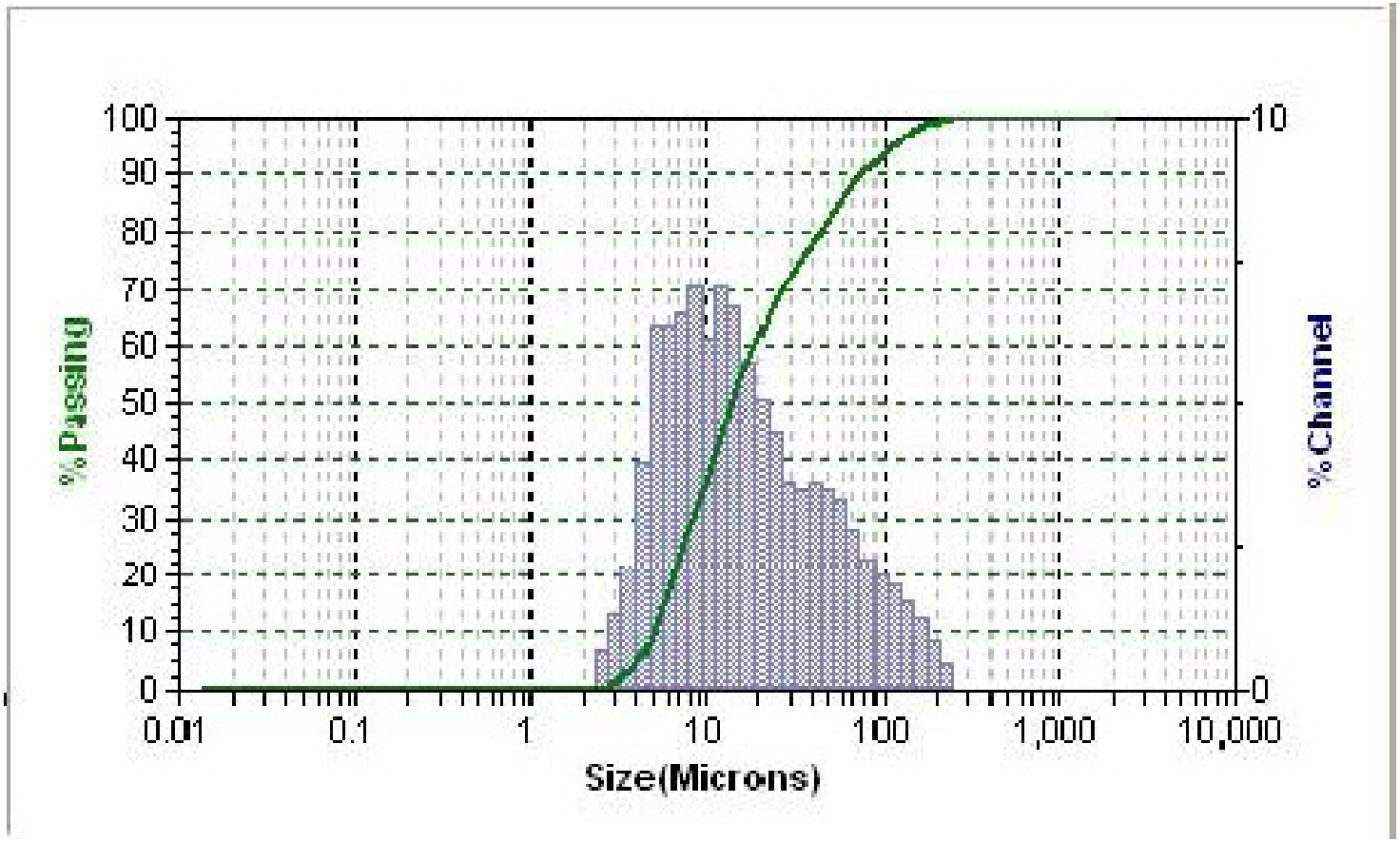


Figure: 15 c.

PARTICLE SIZE DISTRIBUTION F1

(SONICATION TIME= 2 MINUTES)

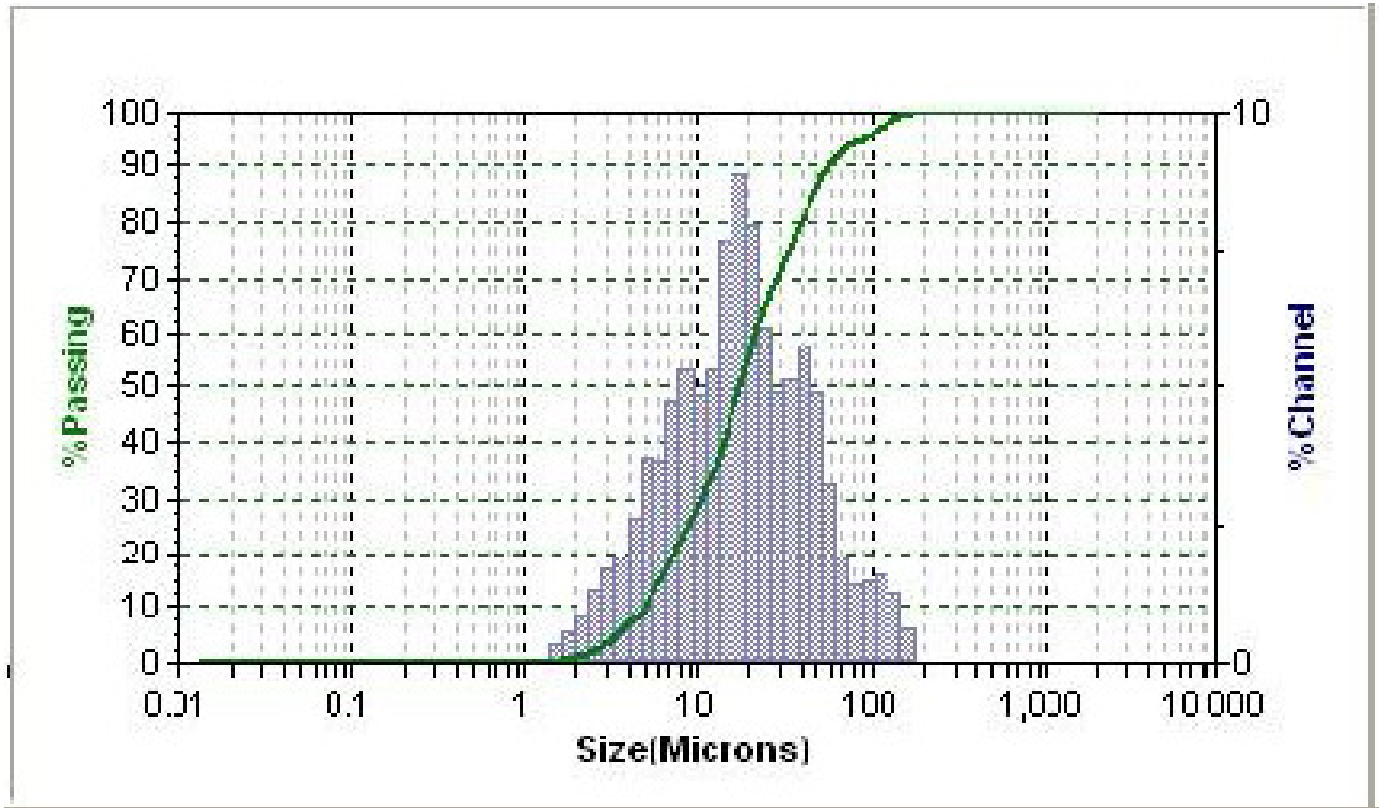


Figure: 15 d.

PARTICLE SIZE DISTRIBUTION F1

(SONICATION TIME= 3 MINUTES)

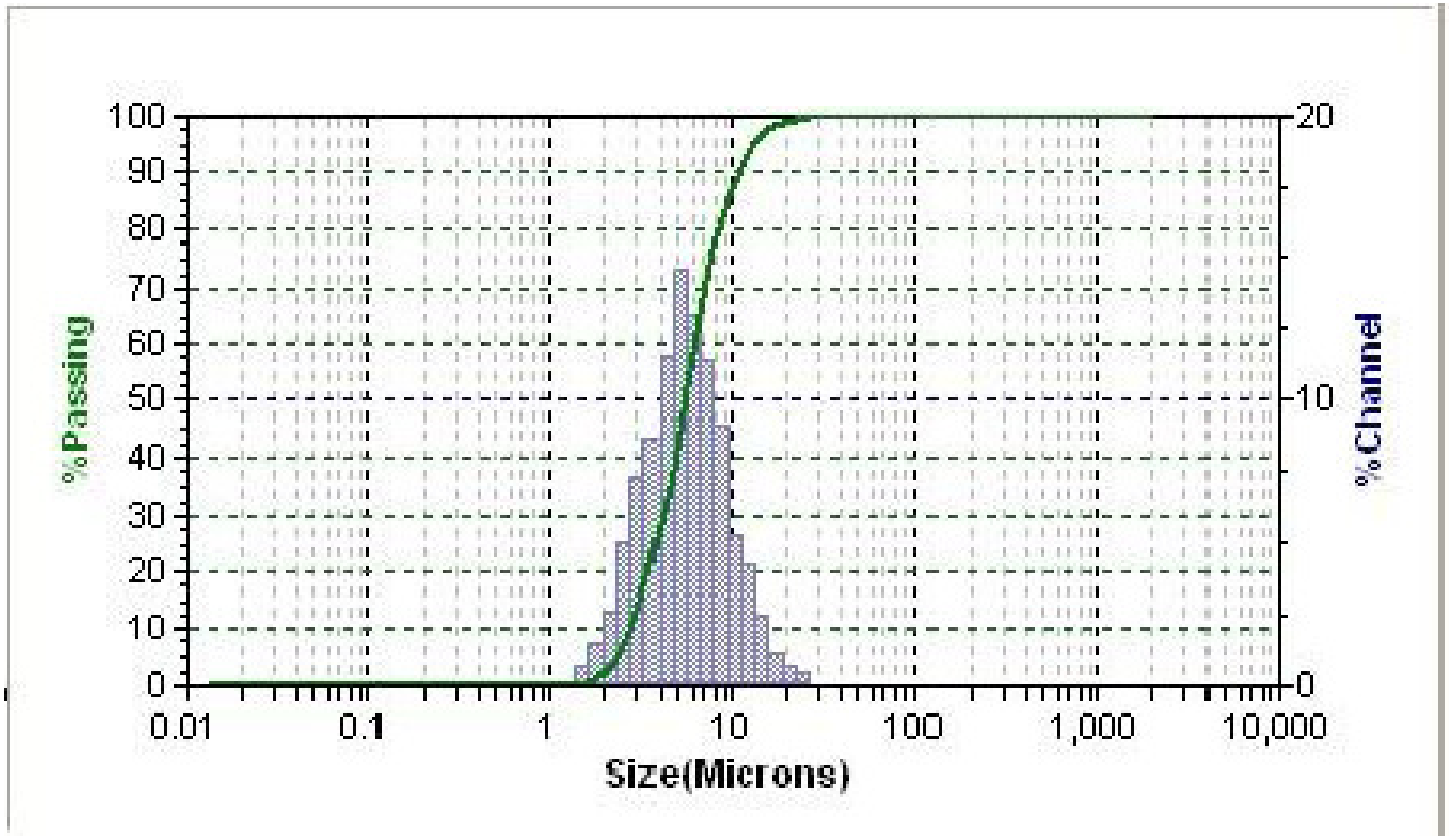


Figure: 15 e.

PARTICLE SIZE DISTRIBUTION F1

(SONICATION TIME= 4 MINUTES)

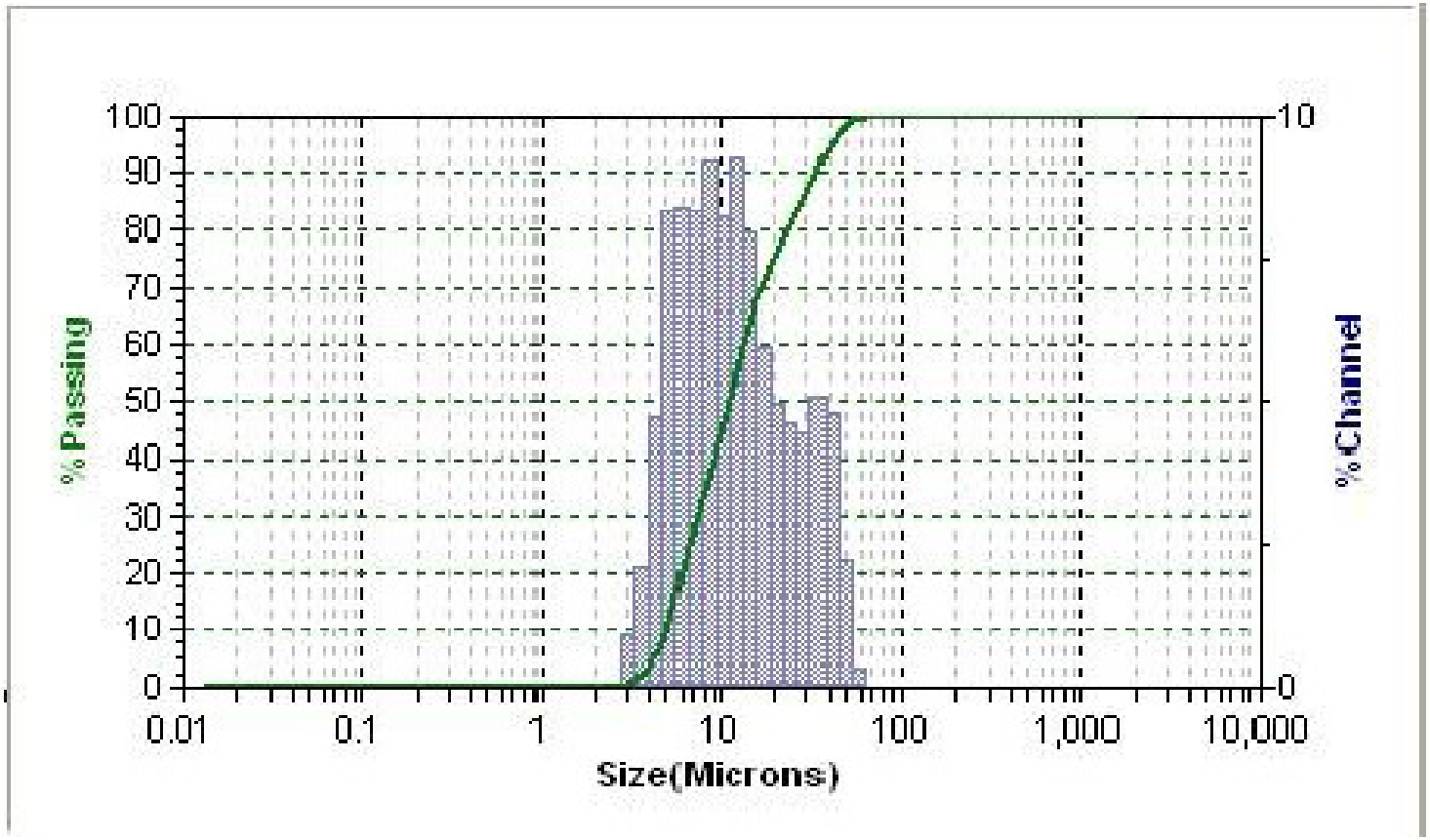
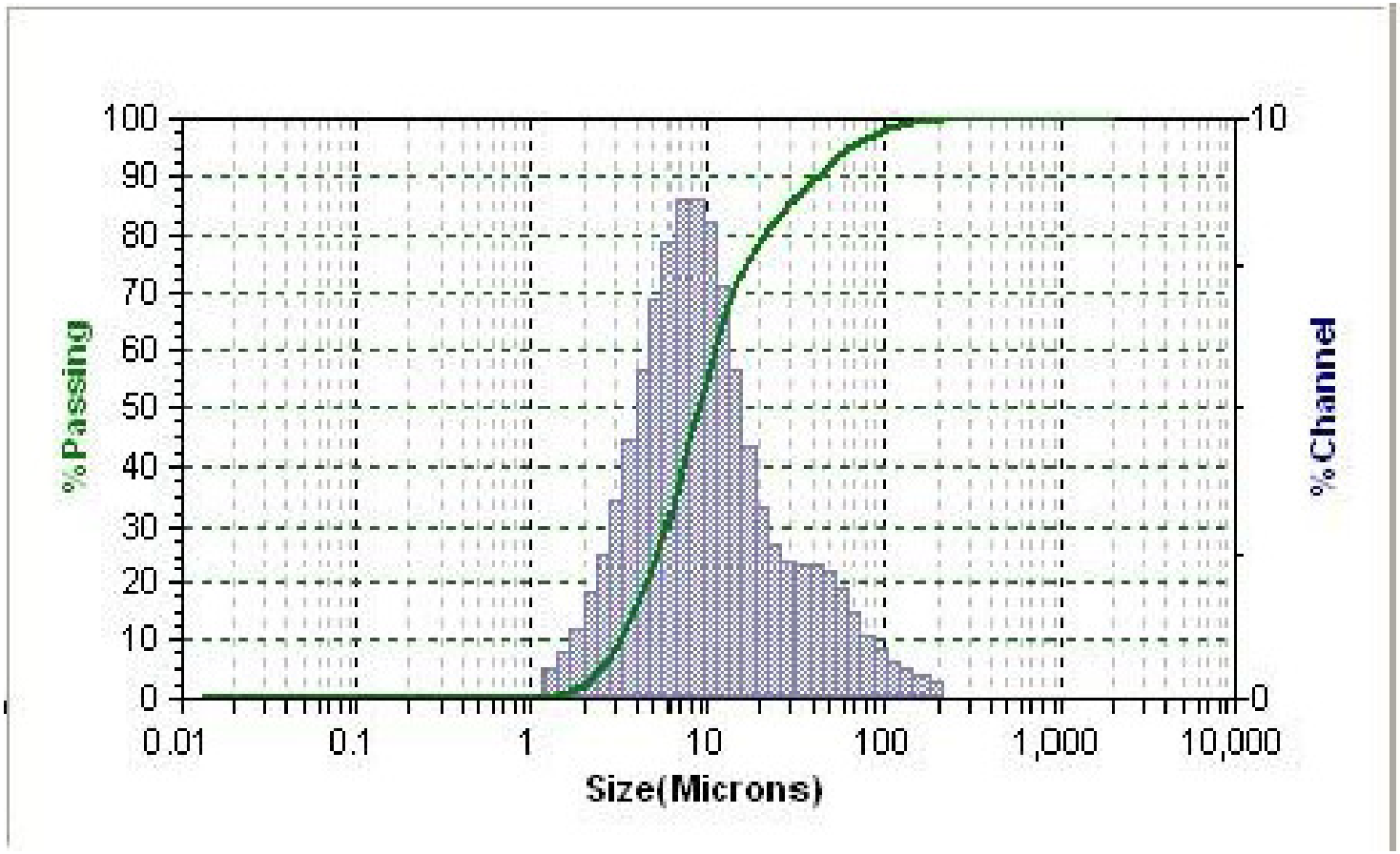


Figure: 15 f.

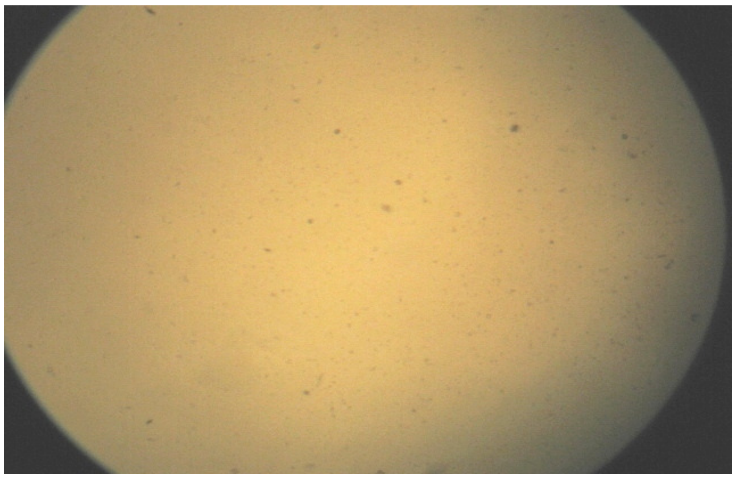
PARTICLE SIZE DISTRIBUTION F1

(SONICATION TIME= 5 MINUTES)

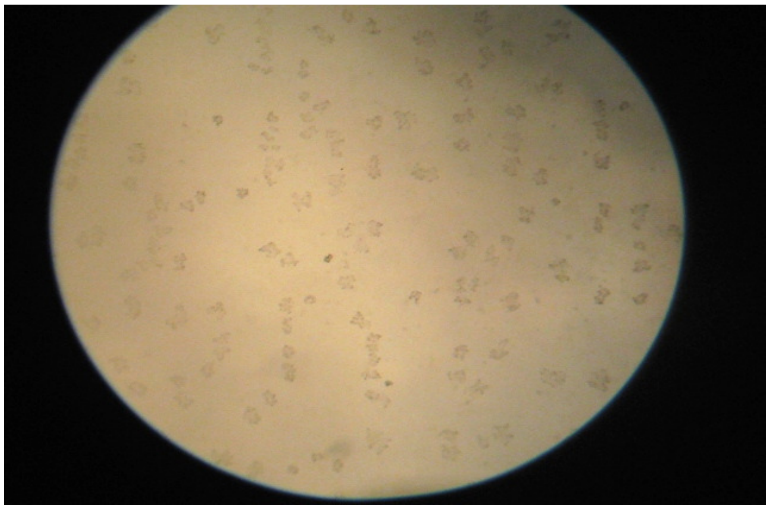


EFFECT OF OSMOTIC SHOCK

[A]



[B]



[C]

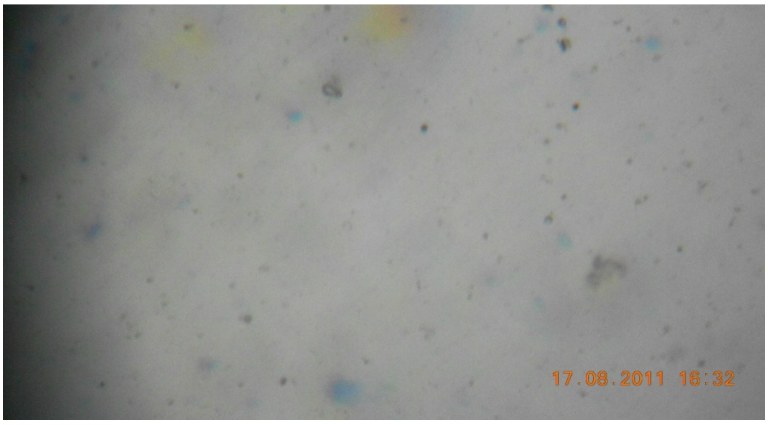


Figure: 16 [A] - HYPERTONIC (1.6% NaCl) [B]- HYPOTONIC (0.5% NaCl)

[C]- ISOTONIC (0.9% NaCl)

Figure- 17

COMPARISON OF INVITRO RELEASE OF RAMIPRIL NIOSOME SPAN 60 1:1 WITH AND WITHOUT CHARGE INDUCING AGENTS.

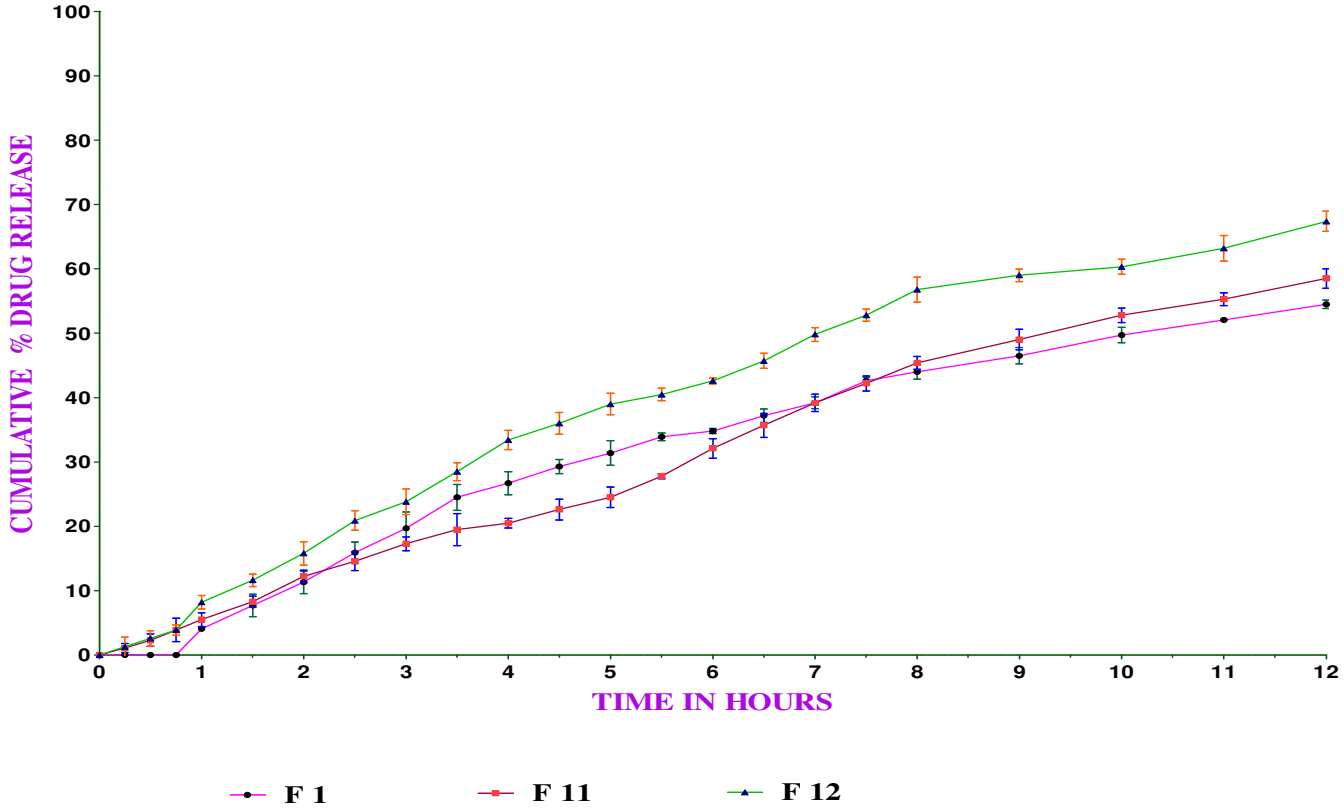


Figure - 18

COMPARISON OF INVITRO RELEASE OF RAMIPRIL DRUG SOLUTION, NIOSOME F1 (SPAN 60 1:1) AND F12 (WITH DCP).

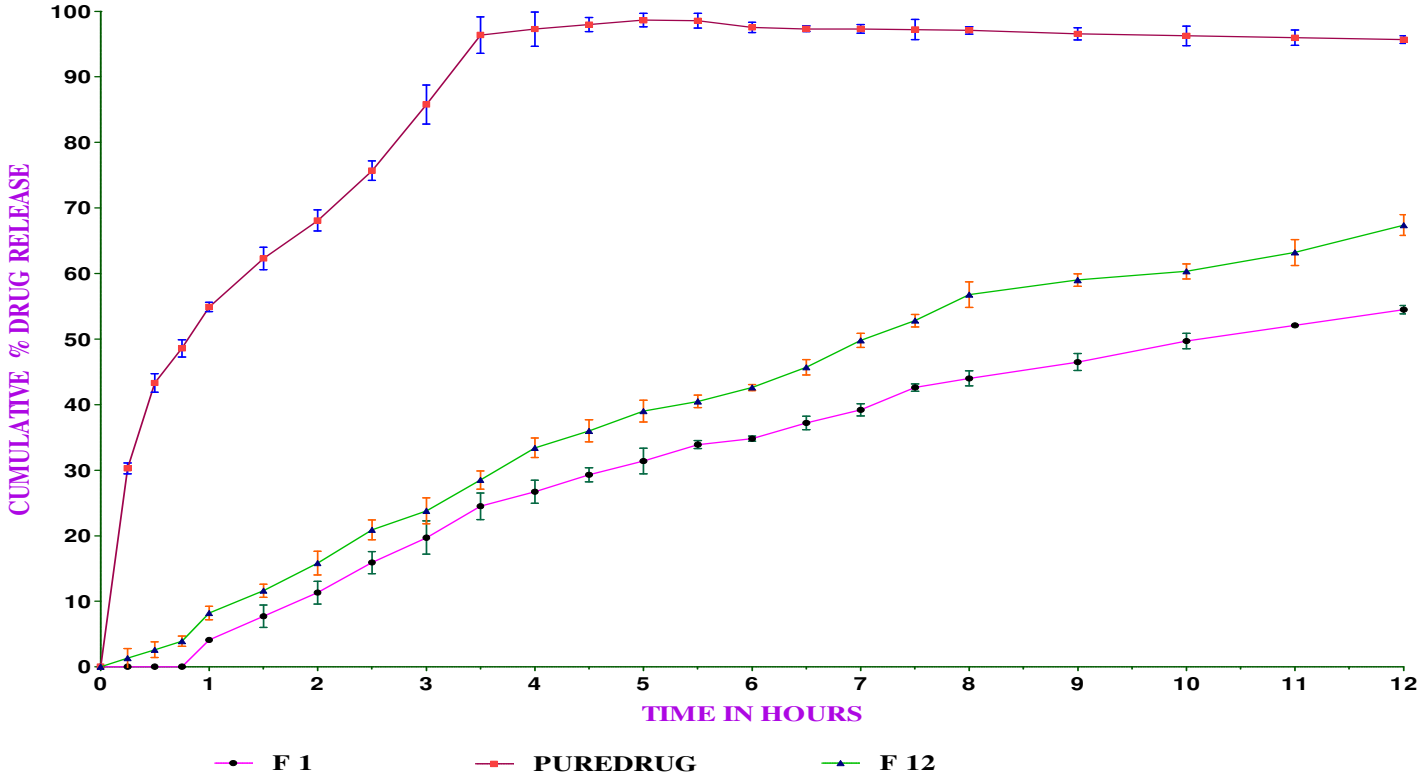


Figure - 19

COMPARISON OF INVITRO *RELEASE* STUDIES OF RAMIPRIL FORMULATIONS

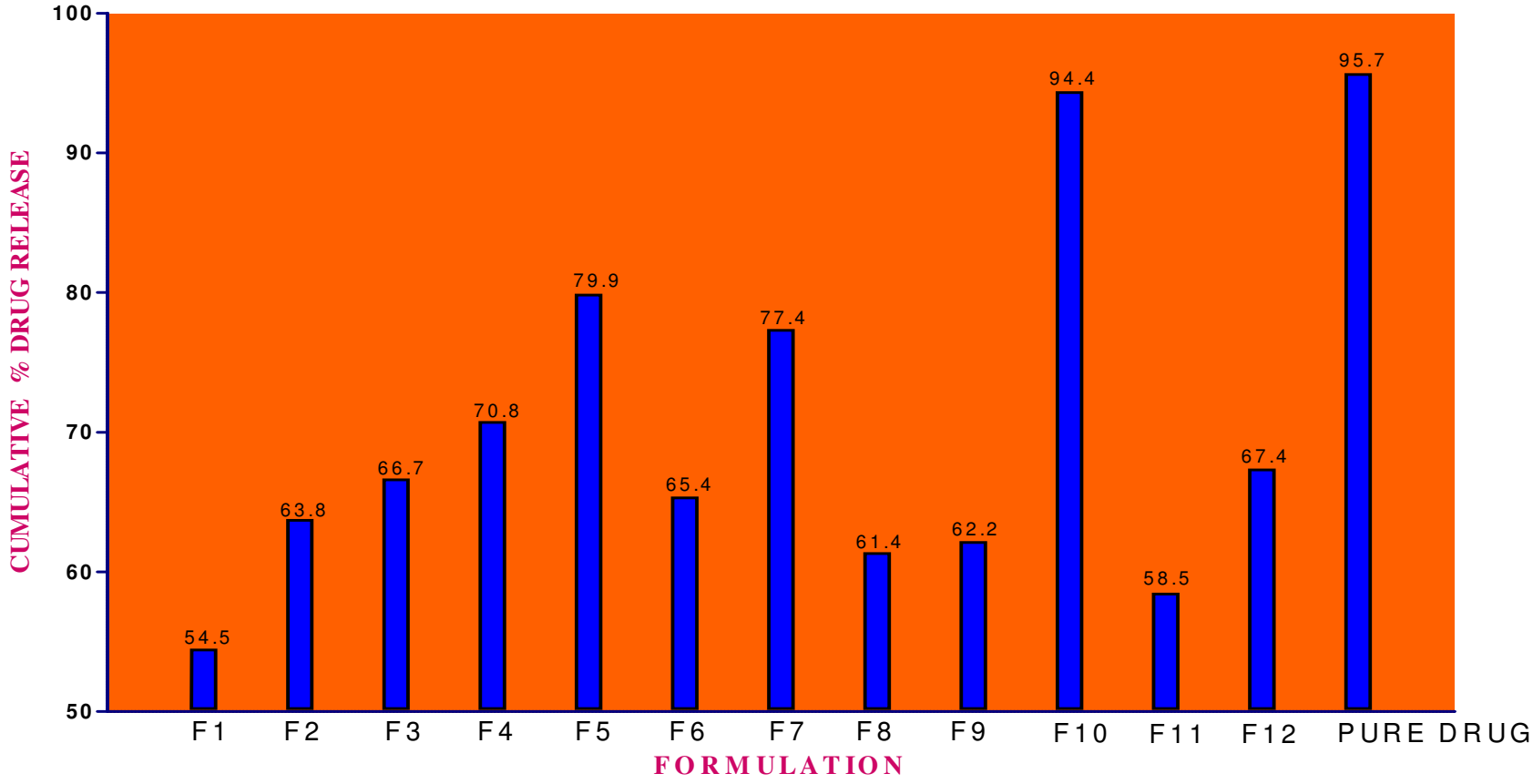


Figure : 20 a.

Particle size distribution (F12) at Malvern zeta sizer.

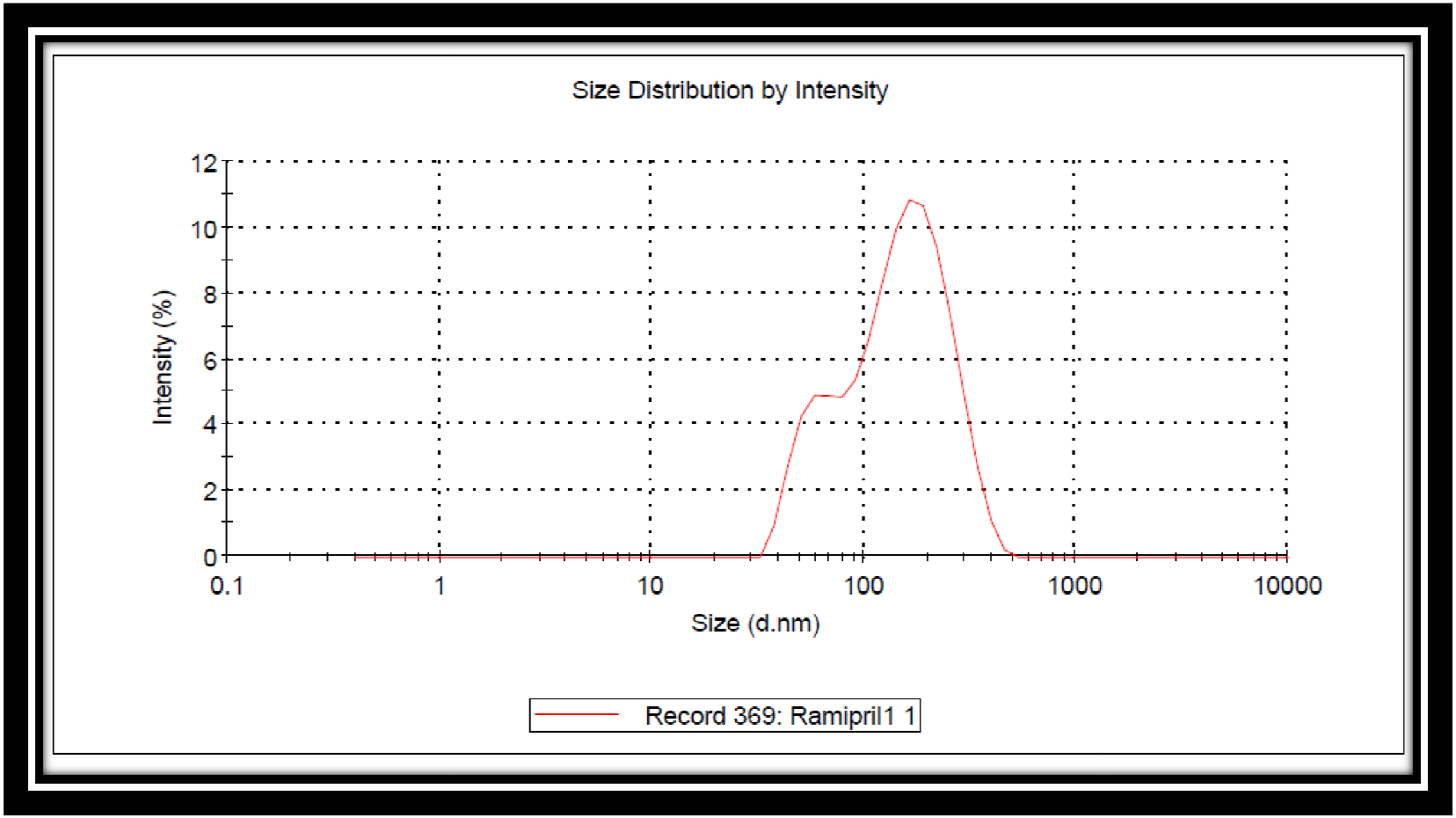


Figure : 20 b.

Particle size distribution (F12) at Malvern zeta sizer.

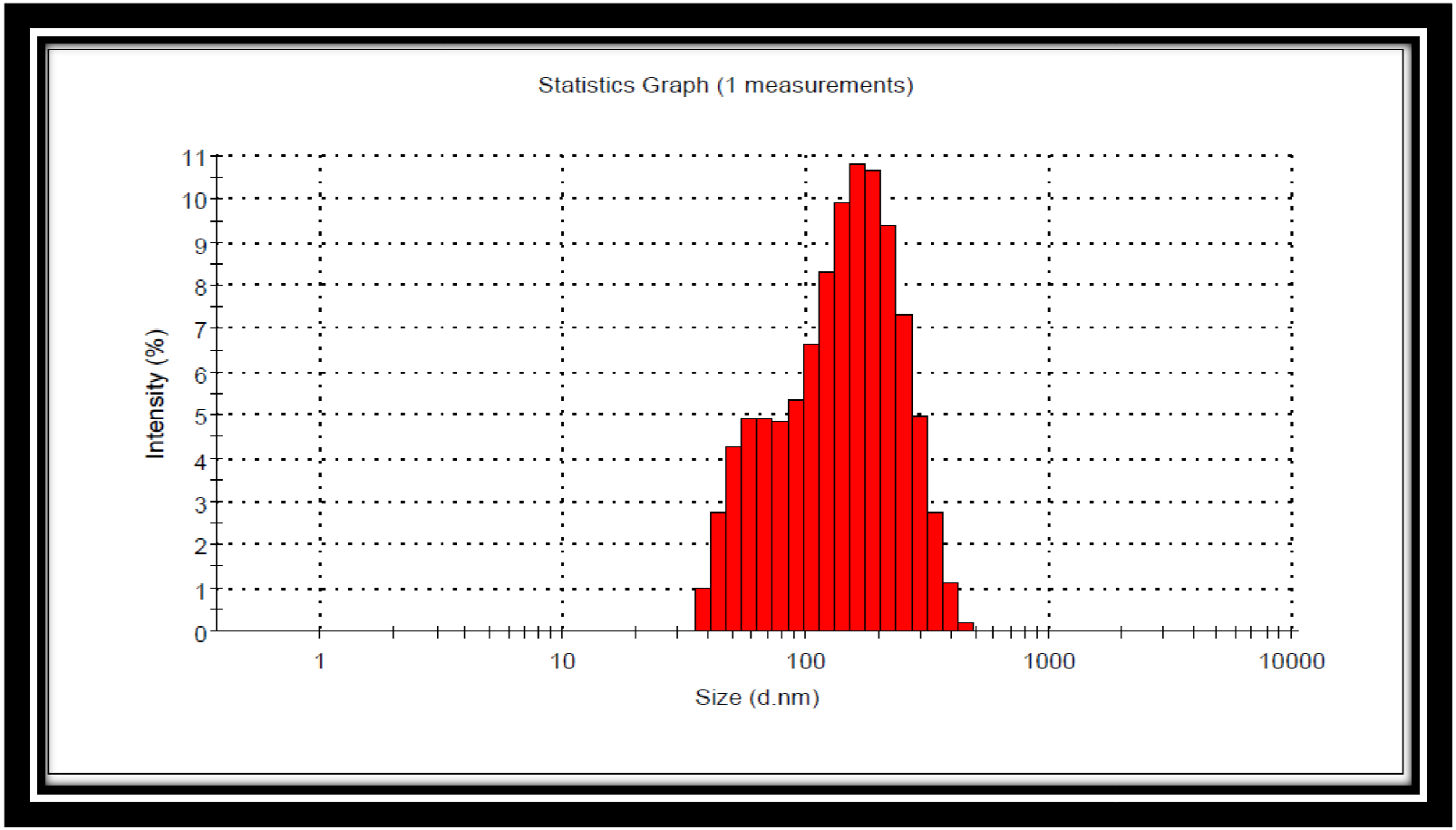


Figure : 21.

Zeta potential mesurement (F12) at Malvern zeta sizer.

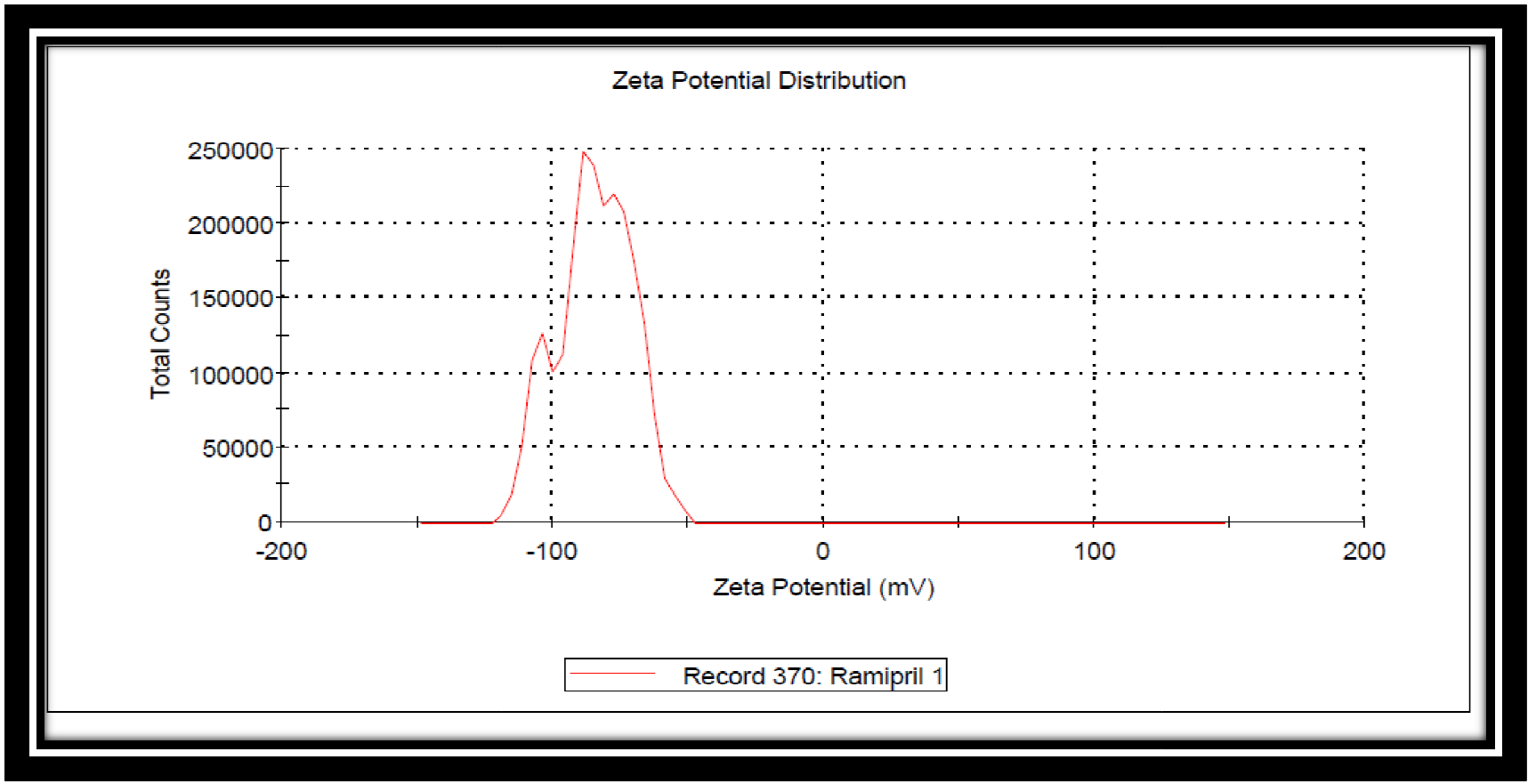


Figure: 22

SEM Photograph of F1

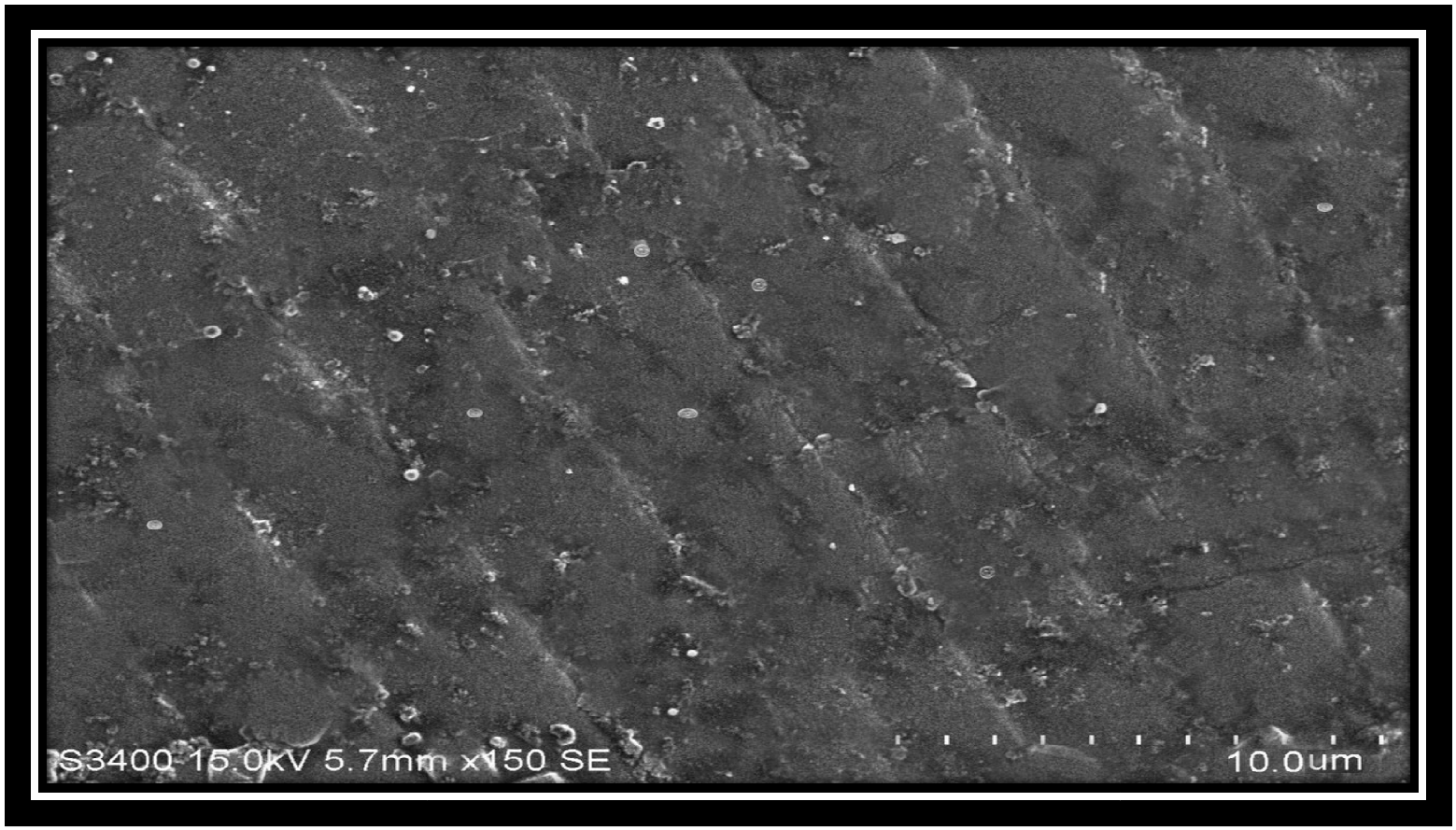


Figure: 23. FT-IR SPECTROSCOPY OF PURE DRUG- RAMIPRIL.

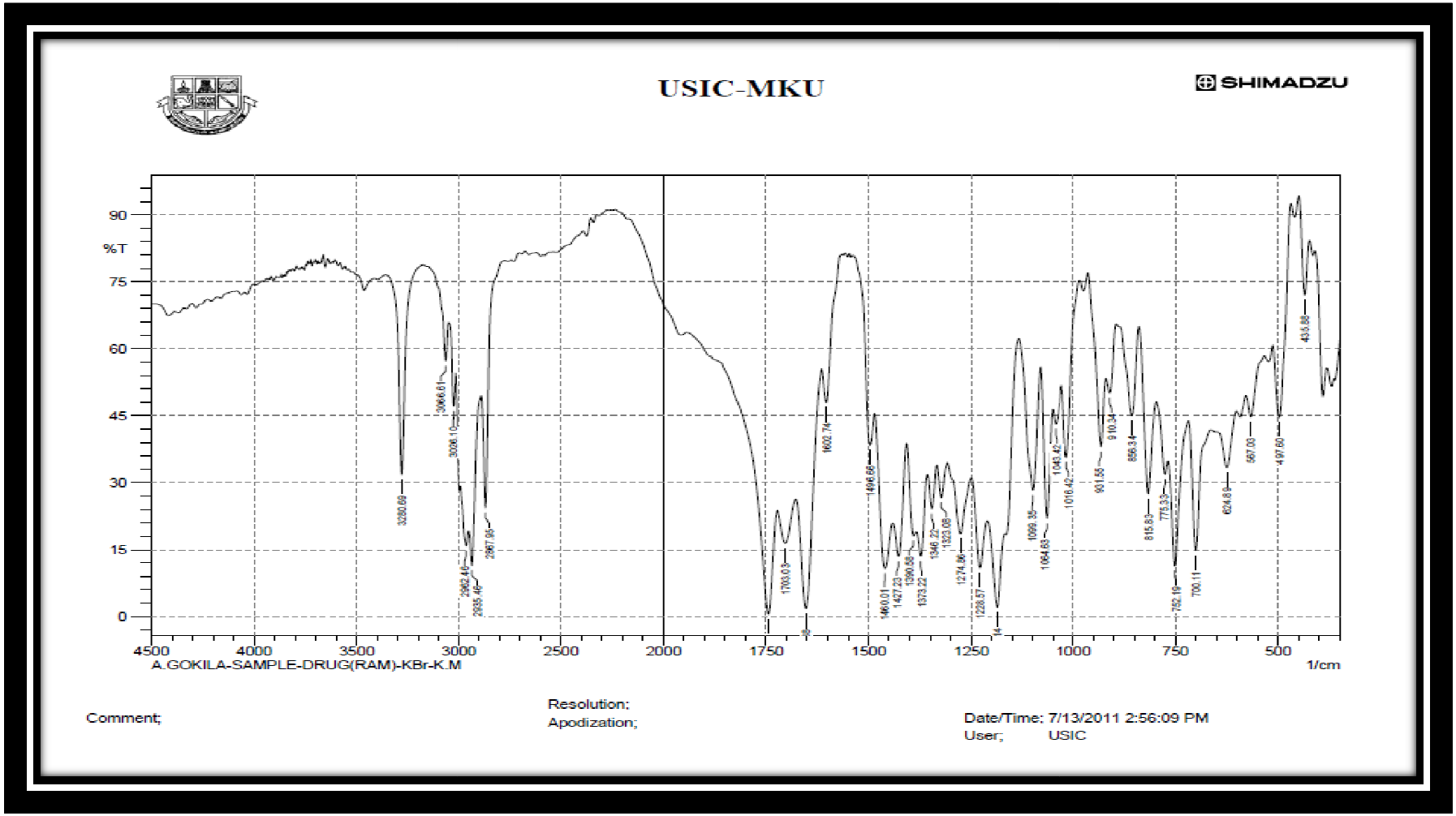


Figure: 24a. FT-IR SPECTROSCOPY OF FORMULATION CONTAINING SPAN 20.

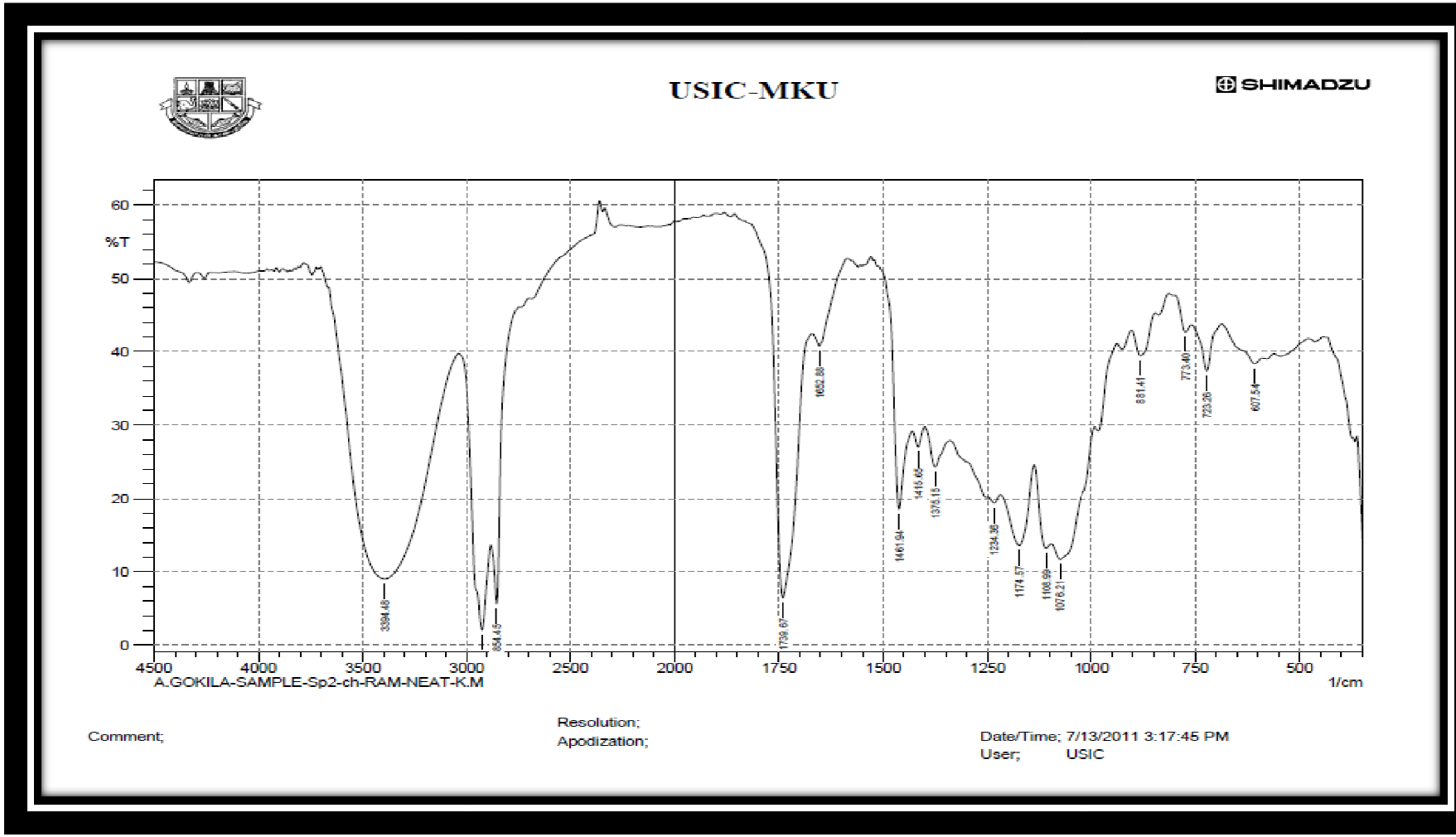


Figure: 24b.COMPARISON OF FT-IR SPECTROSCOPY OF FORMULATION CONTAINING SPAN 20 WITH RAMIPRIL.

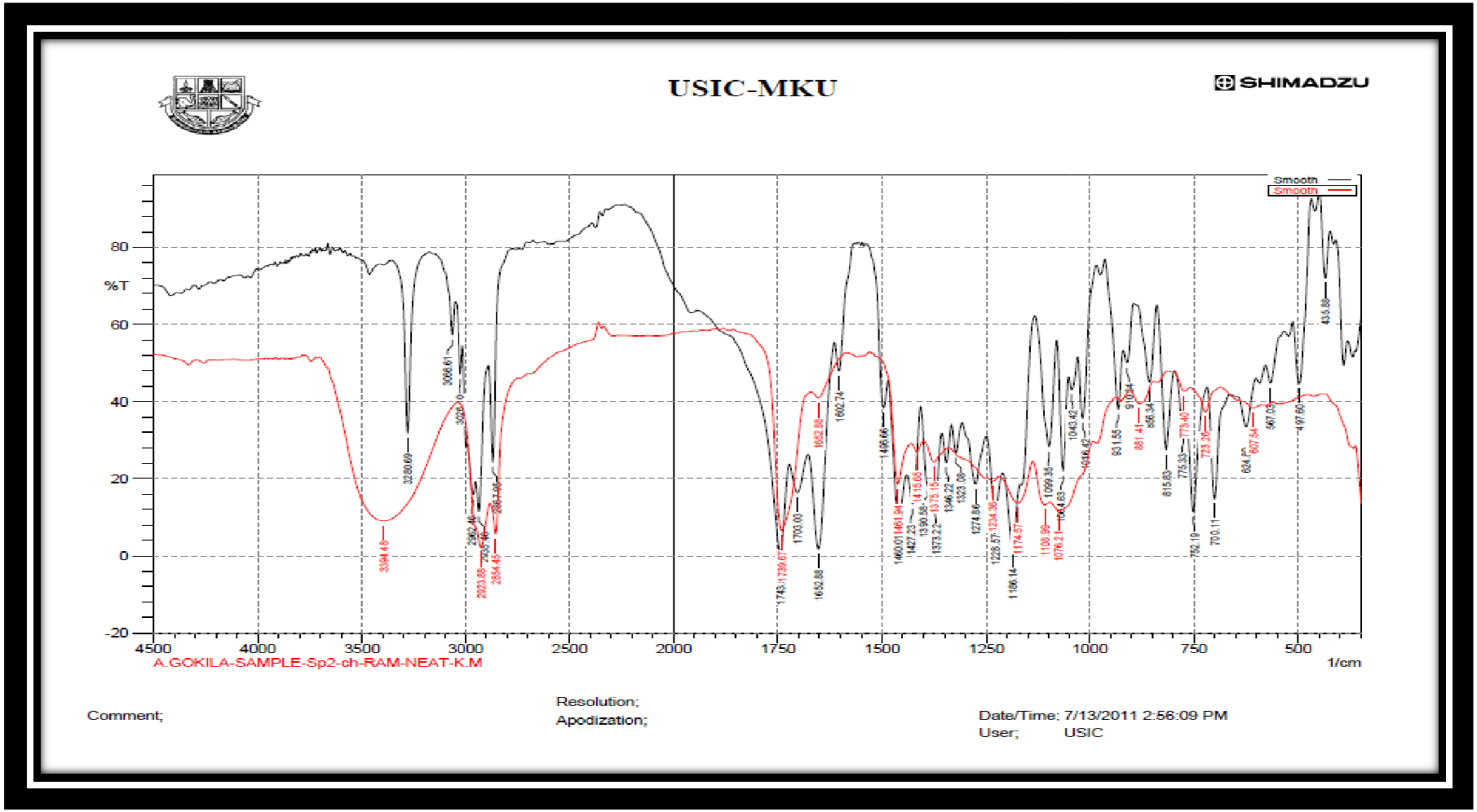


Figure: 25a. FT-IR SPECTROSCOPY OF FORMULATION CONTAINING SPAN 40

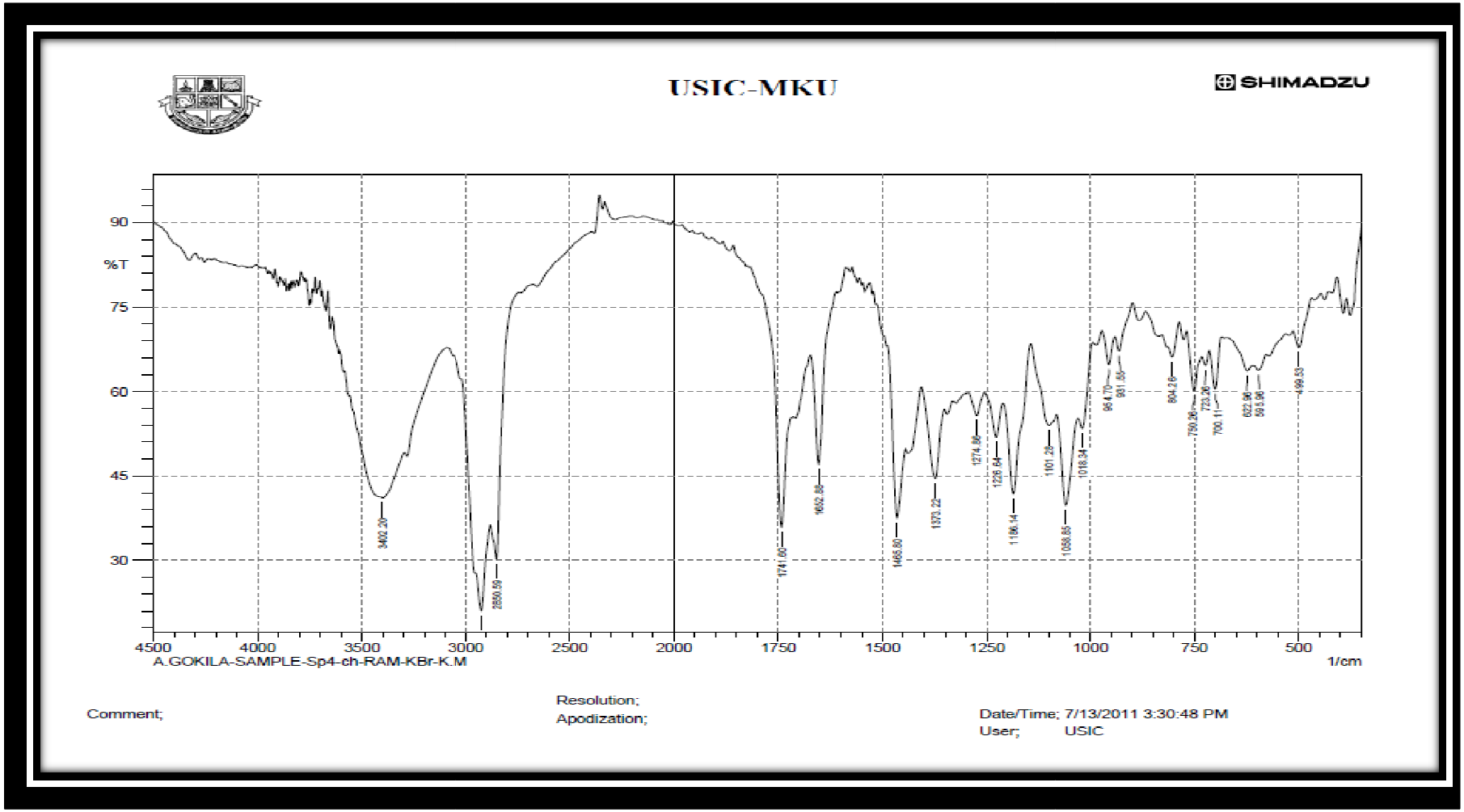


Figure: 25b. COMPARISON OF FT-IR SPECTROSCOPY OF FORMULATION CONTAINING SPAN 40 WITH RAMIPRIL.

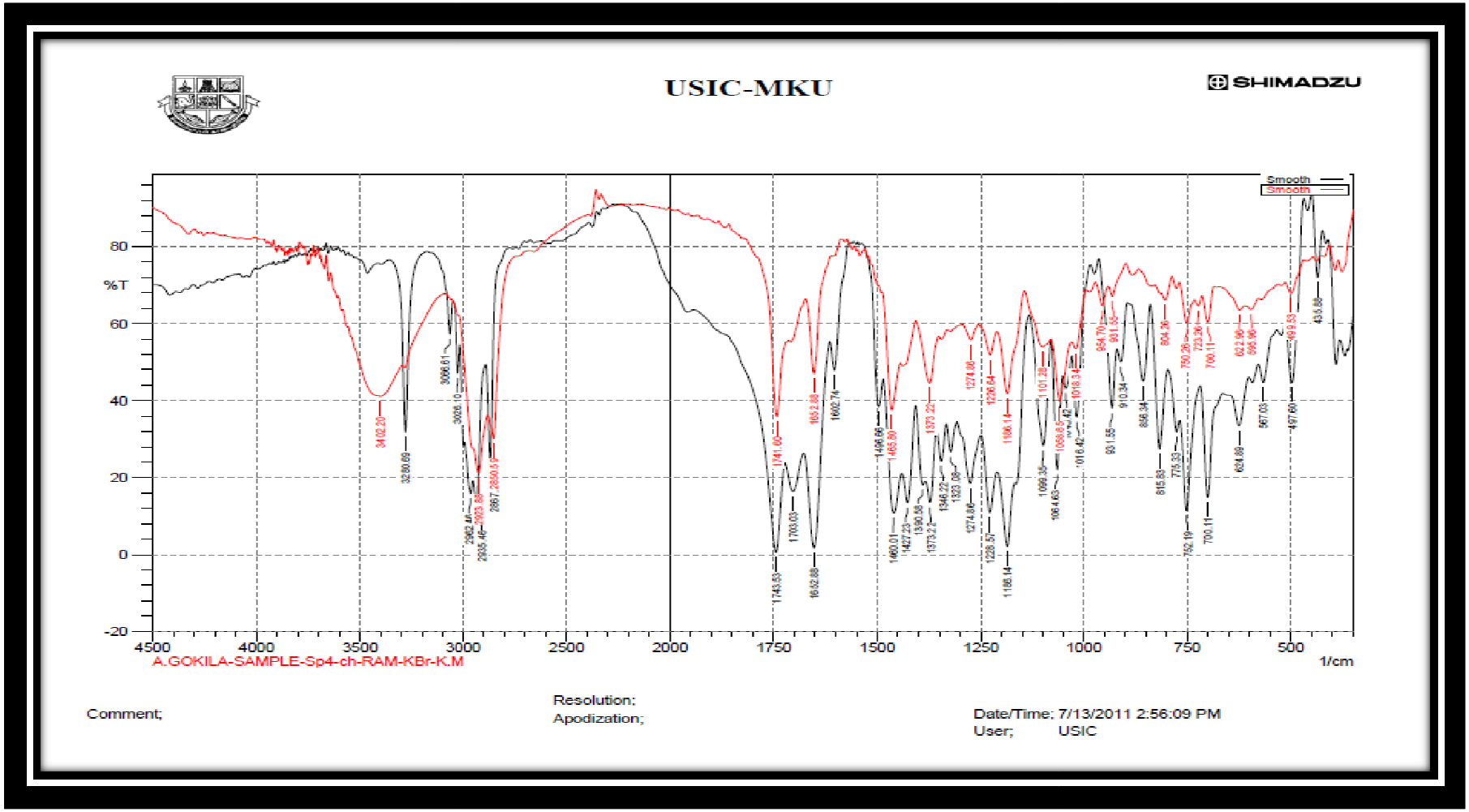
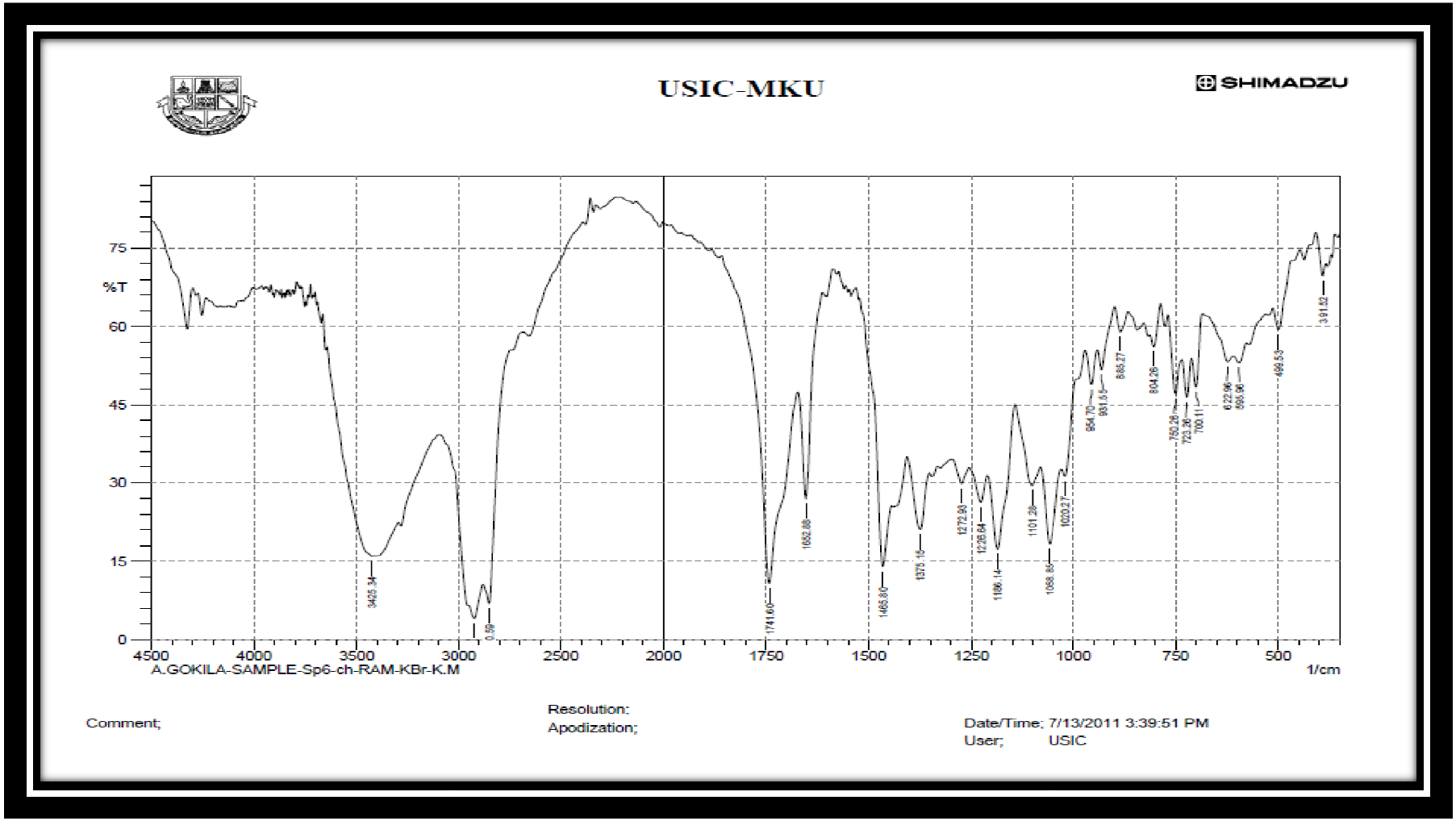
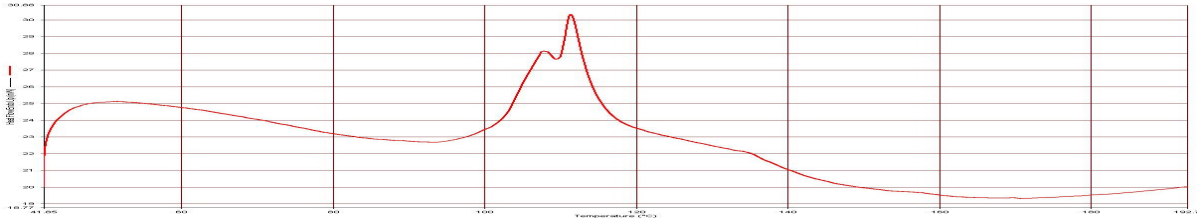


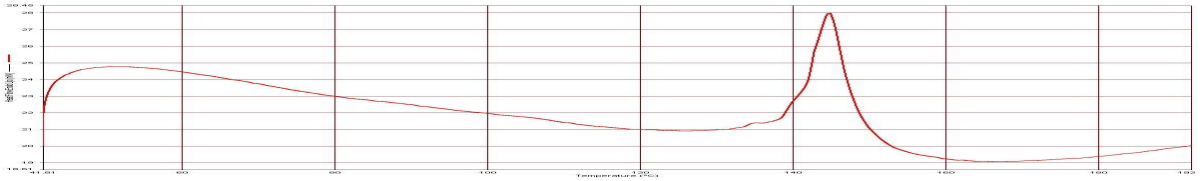
Figure: 26a. FT-IR SPECTROSCOPY OF FORMULATION CONTAINING SPAN 60.



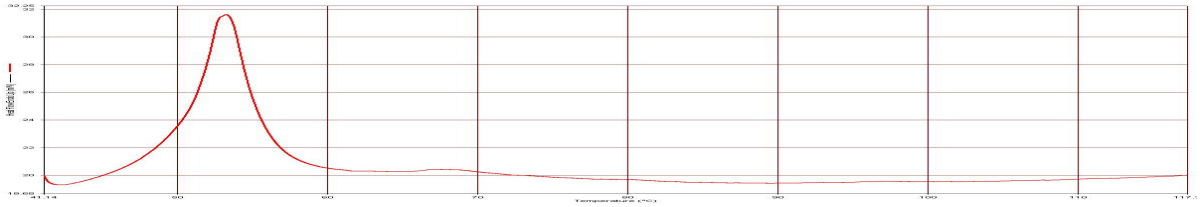
A)



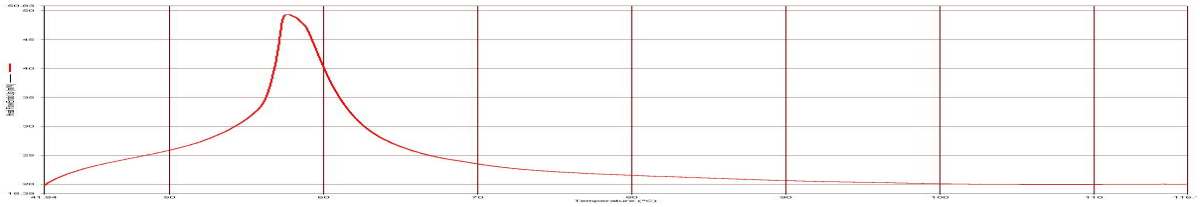
B)



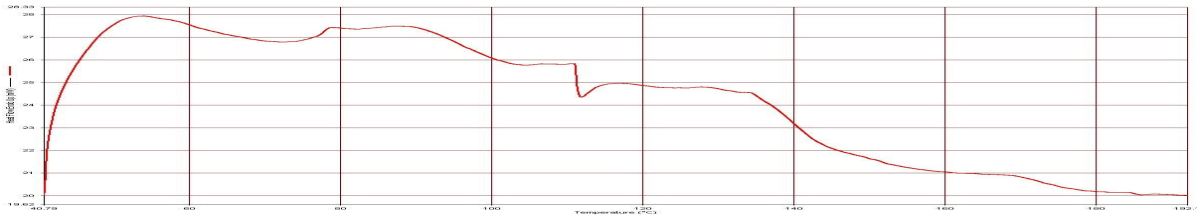
C)



D)



E)



F)

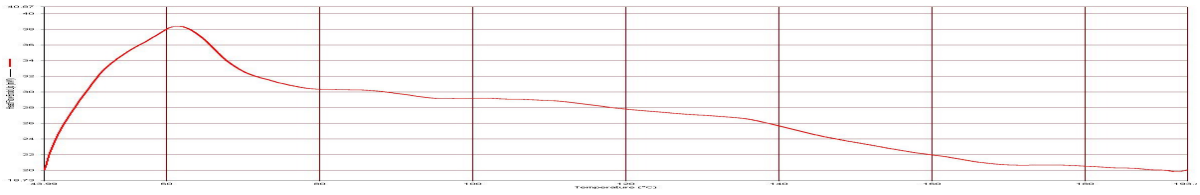
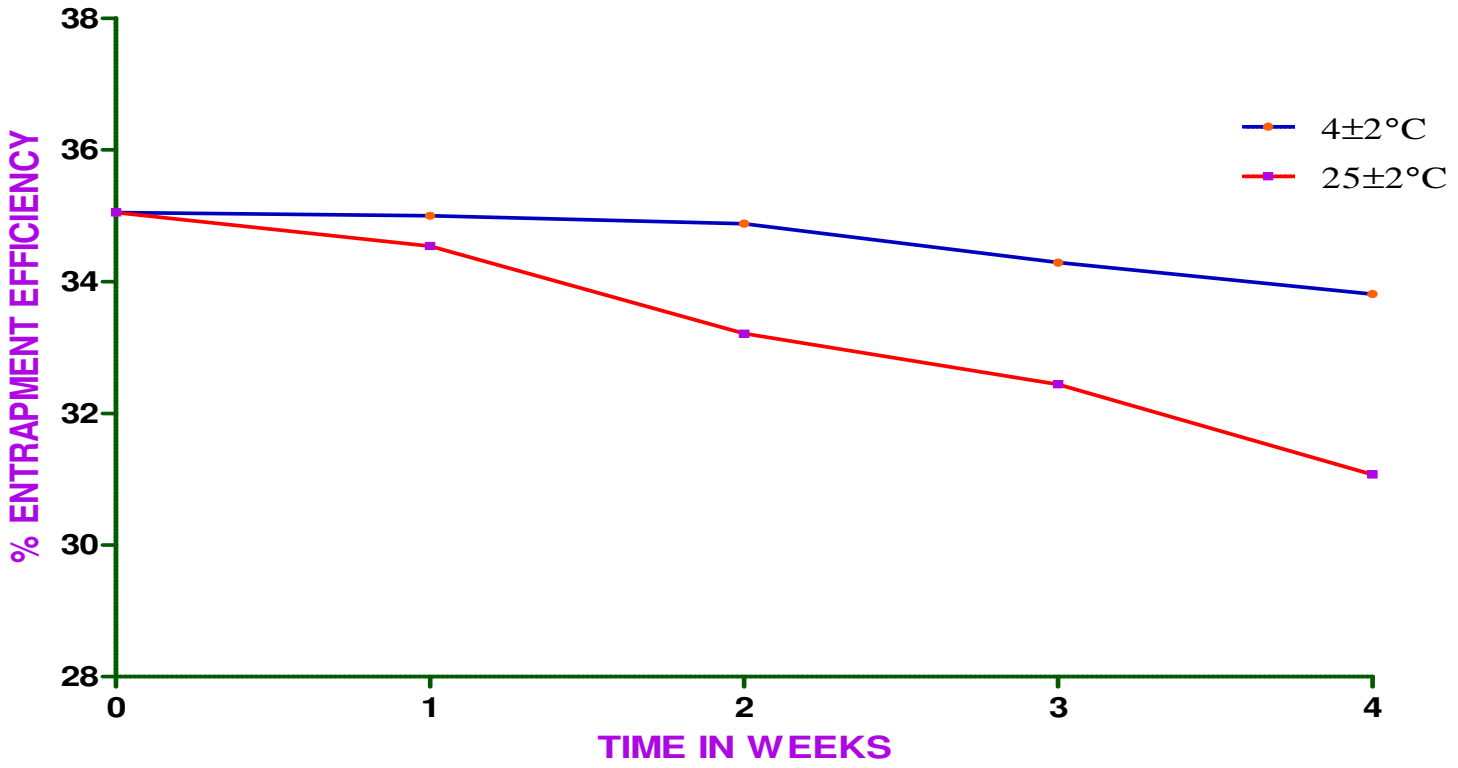


Figure: 28. DSC thermograms are as follows, A) Ramipril; B) Cholesterol; C) Span 40; D) Span 60; E) Formulation containing Span 40 and F) Formulation containing Span 60.

Figure : 29.

STABILITY STUDIES OF RAMIPRIL NIOSOMES AT TWO DIFFERENT TEMPERATURES.



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